COTTON, *GOSSYPIUM HIRSUTUM* L., CULTIVARS DIFFERENTIAL RESPONSE TO SALINITY

A Thesis

by

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ABSTRACT

Arable land for crop production is increasingly affected by soil salinization, including the irrigated land in western and southwestern regions of the United States. The primary water source for irrigated cotton in the Texas High Plains production region is the Ogallala Aquifer, whose depletion has led to increasing amounts of salts in the water and more salt sediment deposited in soil. Research to facilitate identification and development of salt tolerant cotton cultivars was conducted in response to accumulation of salt in soil and water, coupled with decreasing water supply. Germination and hydroponic growth salinity screening methods were evaluated for feasibility in identifying current commercial cotton cultivars that perform relatively well in saline conditions, and in selecting parents for breeders to use for breeding improved cultivars and germplasm.

Thirty-three commercial cultivars, 31 breeding lines and one accession were evaluated for response to salinity in germination experiments at 175mM L^{-1} NaCl concentration and hydroponic experiments at 175mM L^{-1} and 225mM L^{-1} NaCl concentration. Differences were detected among these genotypes in germination percent and hypocotyl length. Cultivars PHY 499 WRF, PHY 367 WRF, and Nitro 44 B2RF germinated relatively well under the imposed salt stress compared with no salt stress conditions. Breeding lines 13-9-218S, 12-18-314V, and 10-B-9 exhibited higher levels of germination among the breeding lines evaluated under salt stress, but not better than cultivar FM 989 and accession TX 65. Evaluations to detect differences among cultivars

and breeding lines for changes in shoot and root length and plant biomass associated with salinity tolerance using a hydroponic system were inconclusive. Germination technique was most feasible for detecting differences in salinity response, but no inference could be made regarding associated salinity tolerance in field production.

DEDICATION

This is dedicated to my wonderful daughter, who provided me with the motivation to continue on this long journey.

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NOMENCLATURE

ANOVA	Analysis of Variance
Cl^-	Chloride
HVI	High Volume Instruments
K^+	Potassium ion
LREC	Texas A&M AgriLife Research and Extension Center at Lubbock
LSD	Least significant difference
Na^+	Sodium
NaCl	Sodium chloride
NCGC	National Cotton Germplasm Collection
NCVT	National Cotton Variety Testing Program
OAP	Ogallala Aquifer Program
OVT	Official Variety Trials
RCBD	Randomized Complete Block Design
RH	Relative humidity
RO	Reverse osmosis
US	United States
UV	Ultraviolet

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a leading textile fiber as well as an important oilseed crop. World-wide, cotton production was estimated to be 30.9 million ha in 2016, down approximately 9% from the previous production year (USDA, 2016). In the United States (US), an estimated 3.6 million ha were planted in the 2016 production year, a 6.2% increase over 2015 (National Cotton Council, 2016). Globally, the US ranks third in cotton production, behind India and China, with approximately 16.5 million bales produced in 2016/17, as compared to the world total of 104.2 million bales produced in the same year (Cotton Incorporated, 2016). The US is ranked highest in the world in cotton exports. Approximately 12.2 million bales were exported in the 2016/17 production year, compared to 35.3 million bales being exported by all other cotton producing countries combined. As an oilseed crop, cotton is ranked third worldwide, behind soybean and corn, with approximately 10-26% of a cotton producer's income coming from the seed. The oil largely is used for human consumption, and the cake that remains after oil extraction is a high protein animal feed used mainly in the beef and dairy industries (National Cottonseed Products Association, 2016). The US produced approximately 37.2 metric tons of oil at a value of US $946.9 million in 2015/16 (USDA, 2016). These collective uses for cotton products contribute to its importance in the US agricultural industry.

Of the estimated 3.6 million ha grown in the US, Texas contributes 2 million ha (National Cotton Council, 2016). The Texas High Plains produces two-thirds of the

Texas production, contributing to 20-30% of the total US cotton production, and 3-5% of world cotton production (Plains Cotton Growers, Inc, 2016). The High Plains is a region characterized by having abundant sunshine, moderate precipitation, frequent winds, low humidity, and a high rate of evapotranspiration (USGS, 2014). Since the late 1880s, agriculture has been the dominant industry in the High Plains region and farmers have irrigated using ground and surface water (USGS, 2014).

For most of the crops grown on the High Plains, irrigation is currently necessary to reach an economically viable yield, and the Ogallala Aquifer has enabled farmers in the Texas High Plains to irrigate. The Ogallala Aquifer is the major groundwater source for the Texas High Plains, and generally is characterized as a fresh water source. The dissolved-solids and chloride concentrations increase from north to south, with generally less than 400 mg/L in the Northern High Plains and exceeding 400 mg/l in the Southern High Plains (Ashworth & Hopkins, 1995). Some areas of the Southern High Plains can exceed 1,000 mg/L, especially near alkali lakes (Ashworth & Hopkins, 1995). The changing climate has increased demand on this valuable water source, forcing water levels critically low in some areas (McGuire, 2014). While not widely used, the Dockum Aquifer is also being considered a potential source of irrigation water for many areas where there is high demand and insufficient capacity in the Ogallala Aquifer. The Dockum Aquifer irrigation water is characterized as generally of poor quality, with water ranging from fresh, dissolved solids of 1,000 mg/l, in the outcrop areas to the east, to brine, 20,000 mg/l, in the confined western parts (Bradley & Kalaswad, 2003; Ashworth & Hopkins, 1995).

Abiotic stresses are major challenges facing crop production that can reduce yield up to 50% (Bray et al., 2000). Approximately 20% of the world's cultivated land and nearly half of the irrigated lands are affected by soil salinity (Wang, 2003; Zhu, 2001). Soil salinization has become a significant issue in the Southwest and Western areas of the United States affecting approximately 23% of the irrigated land (Ghassemi et al., 1995). In areas of the Texas High Plains irrigated by the Ogallala Aquifer, depletion of this valuable water source has led to an increase of salt, including sodium chloride (NaCl), sediment deposited in soil, along with increasing amounts of sodium particles found in water.

Attributed to current climate issues, Wang (2003) suggested that 30% of the arable land world-wide will be affected by soil salinization in the next 25 years. Further, it was suggested that salinity will affect 50% of the total arable land by 2050 (Wang et al., 2003). Salinity resides in the soil's crust for an indefinite amount of time, especially in arid and semi-arid ecoregions.

Crop production will need to be increased by 38% by 2025 and 50% by 2050 to support the growing population (Wild, 2003). The amount of salt concentration contained in water alters many physiological mechanisms within a plant. Salinity affects germination, reduces plant biomass, and slows root growth impacting the nutrients uptake (Munns, 2002). On the Texas High Plains, the accumulation of salt in the soil and water, coupled with a decreasing water supply has become a problem and necessitates an understanding of how to approach identification and development of salt-

tolerant crops. Current cultivars of many of the common crops grown on the High Plains have been developed to perform under intensively managed systems in low stress environments. Plants such as cotton have moderate salt tolerance (electroconductivity (ECe) = 4 to 8 dS m^{-1}), so they are already suited for production in semi-arid climates (Maas & Hoffman, 1977). Many current cultivars have been developed to perform in maximum input environments, and 96% of cotton production in 2014 was from cultivars with high-value genetically engineered traits (USDA, 2014). Current biotechnology resources being used are for herbicide resistance and resistance to Lepidopteran insect species, while other genetic improvement strategies are needed for crops to further adapt to the changing climate. Classical breeding methods incorporate complex, quantitative traits into future crop cultivars, and are important for developing crop cultivars suitable for changing availability and purity of fresh irrigation water. Incorporating abiotic stress-related native traits into crops could also enhance genetic diversity of the crops, allowing them to have the potential for breeding resistance to other abiotic or biotic stressors. Characterizing and measuring abiotic stress response in a breeding program can be complex. Development of a hydroponic salt response screening method could be beneficial, as increased salt tolerance may allow plants to extract more water from the soil in areas where rainfall is low and the salt is concentrated in the rhizosphere (Munns et al., 2006). The objective of the research reported herein was to evaluate the feasibility of salinity response screening methods for breeders to use in germplasm development; and, long-term, to identify methods feasible to develop a salinity response cultivar selection tool

2. LITERATURE REVIEW

2.1 SALINIZATION OF SOIL AND WATER

Salinization is defined as the "accumulation of water-soluble salts within the soil layers above a certain level that adversely affects crop production, environmental health, and economic welfare" (Oxford Bibliographies, 2014). While soluble salts naturally occur in soils and waters, there are other processes that can contribute to the rapid increase of salts in a soil layer including but not limited to: natural weathering processes, fertilizer and pesticide application, dumping of industrial and municipal wastes, other soil conditions that lead to a reduction on leaching of the salts, and numerous other possibilities (Chhabra, 1996). The salinization process is classified as "primary" and "secondary" salinity, based on how salt accumulation occurs. Primary salinization is caused by naturally occurring processes, such as weathering. Secondary salinity is the result of management practices, and has been further classified into three major types of salinity based on the soil and groundwater processes. The classifications are: (1) groundwater-associated salinity, or fluctuation in, usually saline, groundwater leading to water and salt discharge on soil surface layers; (2) non-groundwater-associated salinity, or poor hydraulic properties of the soil layers; and (3) irrigation-associated salinity, where the salts introduced by irrigation water are stored in the soil because of insufficient leaching (Beresford et al., 2001). In arid and semi-arid crop production areas, such as the Texas High Plains, the major source of soil salinization is the result of irrigation.

2.2 SALT-TOLERANT PLANTS

In many areas of the world, many species of plants have evolved mechanisms to tolerate the stress of salinity, as well as drought. These plants, called halophytes, can withstand high concentrations of salt for extended periods of time, and some plants require salt for optimum growth. What is generally considered common table salt (NaCl) is the most commonly considered source of salinity; however, halophytes can tolerate a range of other ions at elevated levels (Flowers et al., 1977). The agricultural potential of halophytes has been reviewed by Mudie (1974), Flowers et al. (2010) and most recently, Ventura et al., (2015).

Salinity tolerance is customarily measured as the percent biomass production in saline conditions versus non-saline over a sustained period (Munns, 2002). Using this method of measurement is considered more reliable for stress response, as salinity often results in changes in the fresh weight: dry weight ratio, as compared to measuring the actual ion concentrations in the sap or other types of measurements (Gorham et al., 1985). Salt tolerance of halophytes or development of more tolerant plants is dependent on several factors. First are the overall environmental factors; temperature, atmospheric humidity, and air pollution have a significant influence on salt tolerance. For example, the same species of plant may seem more tolerant to salt under cool, humid conditions versus hot, dry conditions. Secondly, soil factors, such as soil fertility, water and aeration, can have effects on the salt tolerance of plants. Finally, the growth stage of the plant as well as the cultivar contributes to the salt tolerance (Maas & Hoffman, 1977).

On the plant or variety level the mechanisms of salt tolerance take place at three levels of organization: whole plant, cellular, and molecular (Munns et al., 2002).

2.2.1 Whole Plant Level Salt Tolerance

At the whole plant level, it appears that the main mechanism of salt tolerance is the plant's ability to exclude salt. The physiological mechanism of exclusion on the whole plant, as well as on the cellular level has been discussed in detail by previous scientists (Greenway & Munns, 1980; Lauchli, 1984; Munns et al., 1983; Pitman, 1984; Gorham et al., 1985; Story &Walker, 1999; Jeschke, 1984; Jeschke & Hartung, 2000; Munns, 2002). Salt tolerance of a plant is dependent on the plant's ability to control the transport of salt at five sites: (1) selective uptake by the roots; (2) loading of the xylem; (3) removal of the salt in the upper part of the roots, the stem, petiole or leaf sheaths; (4) loading of the phloem; and (5) excretion through salt glands (Munns et al. 2002). Halophytic plants are the only types of plants that seem to have an effective way to excrete salt from the leaves (Gorham et al., 1985). Most glycophytes, or salt susceptible plants, rely on the first three mechanisms listed above to varying degrees. The genetic variation within a species, or closely related species, has mostly been due to the various degrees of controlling the salt uptake in the roots (Munns et al., 2002). It is also shown that high shoot/root ratios, high intrinsic growth rates, and absence of an apoplastic pathway in the roots contribute to maintaining low rates of salt accumulation in the leaves (Pitman, 1984).

2.2.2 Cellular Level Salt Tolerance

A working theory has arisen in the discussion of the mechanism of salinity tolerance in halophytes at the cellular level. Intracellular ion compartmentation is the widely-accepted hypothesis, and contains three interrelated propositions that are the principle feature of tolerance at this level. This hypothesis suggests: (1) that under saline conditions large quantities of salts, mostly but not limited to NaCl, that are absorbed into the leaves and contribute to the osmotic adjustments are mainly amassed in the vacuole; (2) the concentration of inorganic ions in the cytoplasm is held in the range of 100 to 200 mol m^{-3} and the cytoplasm shows a strong selectivity for potassium over sodium, magnesium over calcium, and phosphate over chloride or nitrate; and (3) under hyperosmotic conditions the maintenance of osmotic equilibrium across the tonoplast requires the accumulation of nontoxic organic solutes in the cytoplasm (Gorham et al., 1985). In short, the plant works to keep the salt out of the cytoplasm and attempts to maintain its accrual in the vacuole. This has been indicated in many species where high concentrations of salts, most commonly NaCl, of over 200 mM, a level that is known to completely repress enzyme activity *in vitro,* have been observed in leaves that are still functioning normally (Munns et al., 1983). If the sodium (Na^+) and chloride (Cl^-) are sequestered into the vacuole, then potassium ion (K^+) and other organic solutes, such as proline and glycine betaine, are believed to accumulate in the cytoplasm to balance the osmotic pressure of the ions in the vacuole (Munns, 2002).

2.2.3 Molecular Level Salt Tolerance

The mechanisms that control Na^+ uptake function by restricting uptake by selective cation transporters and channels, with the addition of the efflux by the antiporter. The antiporter on the tonoplast sequesters the Na^+ in the vacuole (Amtmann & Sanders, 1999). There is no specific Na^+ transporter, but entry is gained by competition with other cations particularly K^+. It is also possible that Na^+ is entering the cell through high affinity K^+ carriers or possibly through low affinity channels called non-selective cation channels. The transporters that maintain low Na^+ levels in other organelles are not known (Munns et al., 2002). The mechanisms controlling Cl^- movement are associated with salt tolerance in some species, and have been reviewed in detail by White and Broadley (2001).

2.3 EFFECT OF SALINITY ON COTTON

Due to the various mechanisms of how plants tolerate salinity, three categories of tolerance have been developed to define a plant's tolerance level (Colmer et al., 2005). Plants can be categorized as low to moderate tolerance (ECe = 2 to 4 dS m^{-1}), moderate to high tolerance (ECe = 4 to 8 dS m^{-1}), and high tolerance (ECe > 8 dS m^{-1}). *Gossypium* species fall into the category of moderate to high tolerance, having been found to have an injury threshold of 7.7 dSM^{-1} (Maas & Hoffman, 1977). Relatively low levels of salt, concentrations of less than 1 dSM^{-1}, have been found to hinder the growth and development of cotton, with the effects becoming more severe as the plants

are exposed for a longer period (Ahmand et al., 2002; Ashraf, 2002; Ashraf & Ahmand, 2002; Chachar et al., 2008; Qadir &Shams, 1977; Razzouk & Whittington, 1997).

Negative effects of salinity can begin immediately following planting by substantially reducing germination and emergence (Hamdy, et al., 1993; Khan et al., 1995; Chachar, et al., 2008; Kent & Lauchli, 1985). It has been shown to significantly reduce primary and secondary root growth, vegetative growth, leaf size and expansion, shoot/root ratio, and stem thickness (Chen et al., 2010; Khan et al., 1995; Reinhardt & Rost, 1995; Wang et al., 2001; Ye, et al., 1997). In addition to the effects on the vegetative growth of plants, salinity has been found to influence the reproductive growth of the plant. Increasing salt concentrations can reduce the number of bolls produced per plant due a higher instance of boll shedding (Chen et al., 2010; Longnecker, 1974). Salinity has been shown to reduce lint percent and fiber quality by reducing fiber fineness, maturity, length, strength and micronaire (Ashraf & Ahmad, 2000; Korkor et al., 1974; Longnecker, 1974).

As the production of fiber is one of the main economic returns of cotton production, the effects of salinity can have significant impacts. While some management practices, such as leaching or surface drainage, can ameliorate the effect of salinity, the introduction of salt-tolerant cultivars could be an effective alternative or complementary option (Bhandari, 2015). As of date, there is not a commercial cultivar classified as salt tolerant for producers in salt impacted areas.

2.4 BREEDING FOR SALINITY TOLERANCE

The possible ways to increase salinity tolerance of crops have been extensively reviewed with Epstein et al. (1980) describing possible technical and biological solutions to the salinity problem. There are two main avenues for improving salt tolerance of any given crop species: (1) searching the natural diversity within the species, or closely related and inter-fertile species, and (2) genetic engineering (Munns, et al., 2002).

Many plant breeding programs have unintentionally narrowed the genetic base and increased genetic vulnerability of many of the world's important crops (Campbell et al., 2010). Introgression of the genetic resources that are stored in various germplasm collections could provide the benefit of broadening the base of genetic diversity within various crops. Due to the complexities of salt-tolerance mechanisms within plants, efforts ranging from standard hybridization to molecular marker assisted selection have produced few salt-tolerant cultivars (Witcombe et al., 2008). The use of conventional breeding methods has been reported to be slow in development and found to be not as effective as originally hoped. With the advancement of DNA technology, it has been determined that the direct integration of quantitative traits could be a possible option to develop and release salt-tolerant cultivars (Joshi et al., 2015). The use of conventional breeding methods and molecular markers will require extensive and precise screening, phenotyping, and validation strategies to be successful.

2.5 SCREENING FOR SALT TOLERANCE

Evaluation of salt tolerance has been reported for many agronomic crops, with most of the agronomic crops being classified for salt-tolerance, most notably by Maas and Hoffman (1977). Screening for salinity has been most extensively discussed in wheat and other cereal crops, due to the economic importance of cereals as a food staple across the world. Munns (2006) extensively reviewed the various approaches that have been used to increase salt-tolerance in wheat and cereal crops, including barley and rice. In screenings for salt tolerance of cereal, more focus has been on identifying genetic sources that give reduce of Na^+ accumulation into leaves, with focus being less on tolerance of high Na^+ concentrations which is more difficult to quantify. These authors also found that traits that improve the osmotic effect of salt outside the roots, such as morphological or developmental patterns that conserve water, could provide greater effect on growth and yield when compared to the salt-specific components of tolerance. Large gains from the use of inter-species diversity were created by selecting for specific traits, then recombining through a donor parent rather than selecting for individual tolerances *per se.* (Munns et al., 2006). The use of genetic transformation was expected to provide new and useful genetic material. Colmer et al. (2006) reviewed the use of wild relatives, specifically wheatgrass species, to improve tolerance in wheat. In barley, where little is known about the mechanisms of tolerance, one of the most recent studies was conducted by Tavakkoli et al. (2012). This study screened 60 barley genotypes for salt-tolerance and uptake of Na^+, Cl^-, and K^+. A second screening of 15 selected genotypes was conducted using a combination of hydroponic and field methods.

Authors found that salt exclusion and osmotic tolerance are involved in salt-tolerance, and the importance of these traits varies depending on the environment. In horticultural crops, relations between salinity and mineral nutrients have been studied and compiled by Grattan and Grieve (1999). Authors reviewed studies conducted in various conditions: field vs greenhouse, soil vs hydroponic, varying environmental conditions; and long versus short term studies. They concluded that salinity has a direct effect on nutrient uptake, and that there is the possibility of improving some of the symptoms of salinity stress with nutrient additions, such as foliar or soil applications of Ca^{2+} (Grattan & Grieve, 1999).

In cotton, screening for salt-tolerance has been a constant effort for many years, often with results mimicking those in other crops. One of the earliest attempts in screening cotton for salt tolerance was conducted by Abul-Naas and Omran (1974), finding that *G. barbadense* was more tolerant to elevated levels of salinity than *G. hirsutum*. In 2002, two extensive critical reviews of various screening methods and their results were published by Ashraf (2002) and Ahmand et al. (2002). In both reviews, effects of salinity on germination and emergence, root growth, shoot growth, seed cotton yield, fiber quality, seed oil content, and molecular components of salt-tolerance in cotton were comprehensively examined. Both studies found that high salinity levels inhibited cotton's growth and seed yield, but there are conflicting results regarding whether tolerance changes throughout growth or if tolerance was consistent throughout growth. They found that there is a high inter- and intraspecific variation for salt-tolerance in cotton, and that growth, yield, and fiber traits have a significant additive

genetic component of variation, both of which can be easily exploited through selection and breeding programs (Ashraf, 2002). The most recent research on this topic has mainly been a continuation of the studies reviewed by Ashraf (2002) and Ahmad et al. (2002). Reductions in germination, emergence, stand establishment, shoot/root ratios, yield, lint percent and fiber quality were found in more recent studies (Basal et al., 2006; Chachar et al., 2008; Dodd et al., 2010; Wang et al., 2001, Sattar et al., 2010; Chen et al. 2010; Higbie et al., 2010; Abbas et al., 2011; Akhtar et al., 2010; Aftab et al., 2015; Bibi et al., 2016; Muhammad et al., 2015).

While there are extensive studies published on cultivars and breeding stock, screening feral or non-cultivated germplasm accessions available in germplasm collections are limited. Basal et al. (2006) evaluated 20 converted race stock (CRS) cotton accessions for response to salt stress to identify salt-tolerant CRS accession(s) or individual plants within the accessions for potential parent material. Researchers discovered three CRS lines that provided possible positive attributes for use as parent material for breeding salt-tolerant upland cotton cultivars. Akhtar et al. (2010) used 12 cotton genotypes from the Ayub Agriculture Research Institute, in Faisalabad, Pakistan to examine the generally used screening methods for salt-tolerance, i.e., (1) seedling-based, solution culture method and (2) plant yield-based, soil method. This research determined that solution culture screening methods were equally successful for both selection and recognition of salt-tolerant genotypes and that this method was effective for selecting genotypes to transition into field testing. Basel (2011) evaluated five upland genotypes, finding three with moderate to high tolerance. Abbas et al. (2011) assessed

50 genotypes in different salt concentrations, and were able to identify six genotypes as tolerant. Authors also supported conclusions provided in Ashraf (2002) that traits for salt tolerance have a moderate to high genetic variability and are highly heritable traits. Castillo (2011) used a hydroponic method in an attempt to evaluate 209 wild and primitive TX accessions as an agronomic model. Castillo found TX 307 and TX310 had relatively positive responses. It was found that the hydroponic technique used in these studies was an effective method to find individual plants with positive morphological and physiological responses to salinity stress. Bhandari (2015) evaluated 150 CRS accessions in hydroponic and pot based methods, finding four CRS lines that performed well in both systems. Most recently, Bibi (2016) screened eight cotton genotypes at five NaCl concentrations, finding highly significant differences among genotypes for stem diameter; leaves per plant; root length; fresh shoot and root weights; and dry shoot and root weights. This base of knowledge from past research suggests that for development of salt tolerant cotton cultivars to move forward, screening methods must be refined to the point that large amounts of germplasm can be screened producing repeatable and therefore reliable results. Once identified, any resistant germplasm can be integrated into phenotypic breeding programs.

3. MATERIALS AND METHODS

3.1 PRELIMINARY TESTING

A continuing series of hydroponic studies have been conducted at the Texas A&M AgriLife Research and Extension Center at Lubbock (LREC) since April 2006 (Castillo and Dever, 2010). Feral cotton accessions of various origins were screened for salt tolerance using a hydroponic system as an agronomic model to address salinity stress. More than 200 accessions from the National Cotton Germplasm Collection (NCGC) were screened, and TX 307 and TX 310 were identified as having relatively positive response to salinity stress. Knowledge and infrastructure gained from these experiences were used to conduct studies examining the effects of salinity on germination and root biomass development in current commercial cotton cultivars and potential breeding lines.

Preliminary trials starting in 2012 were conducted to modify the hydroponic system from identifying halophytic accessions from the NCGC to detect response differences among current commercial cotton cultivars. Five salt concentrations and five development stages were evaluated to optimize the salt concentration and timing needed to detect critical differences among cultivars. Measurements included germination percent, radicle length, fresh root weight, dry root weight, fresh shoot weight, and dry shoot weight. Hydroponic trials were conducted in 2013 and 2014 at the LREC greenhouse complex. Cultivars evaluated were the 35 commercial or near commercial cultivars entered into the 2013 Official Variety Trials (OVT) in the Plains region of the

National Cotton Variety Testing Program (NCVT); current commercial or near-commercial cultivars that are most often used by producers in the region. Preliminary data analysis was conducted for a separate Ogallala Aquifer Program (OAP) project tasked with evaluating cultivars entered in the Plains region of the NCVT. Since only eight lines can be evaluated at the same time in the hydroponic system, a reoccurring check, FM 989 (PI603956, PVP9800259), was used in each experiment in an attempt to normalize data between experiments.

3.2 GERMINATION TESTING

The first phase of testing was evaluating germination rates and hypocotyl length reduction under salinity stress. The method for screening was modified from Hemphill et al. (2006); salinity levels in this method were either too high or too low, resulting in high plant mortality or un-detected differential responses. Preliminary testing showed improved comparison was achieved using an intermediate salinity level. Cultivars evaluated were those entered into the 2013 and 2014 OVT in the Plains region of the NCVT; 32 and 35 current commercial or near-commercial cultivars that are most often used by producers in the region, respectively. Proprietary breeding lines evaluated were developed in the LREC Cotton Improvement Program; lines included in advanced and intermediate strains testing in 2014 (Table 1). FM 989 was used as the reoccurring check.

Table 1. Cotton accession and LREC breeding lines* evaluated for response to salinity stress during germination, 2015.

Designation	Origin	Breeding Objective
TX 65	NCGC	N/A
6-21-519 FQ	LREC Breeding Program	Fiber Quality
6-46-153 P	LREC Breeding Program	Abiotic Stress
10-B-9	LREC Breeding Program	Fiber Elongation Research
10-B-10	LREC Breeding Program	Fiber Elongation Research
10-B-11	LREC Breeding Program	Fiber Elongation Research
10-B-12	LREC Breeding Program	Fiber Elongation Research
11-11-307 BB	LREC Breeding Program	Bacterial Blight
11-11-505 BB	LREC Breeding Program	Bacterial Blight
11-11-607 BB	LREC Breeding Program	Bacterial Blight
11-18-128 N	LREC Breeding Program	Root-Knot Nematode
12-1-609 FQ	LREC Breeding Program	Fiber Quality
12-1-640 FQ	LREC Breeding Program	Fiber Quality
12-1-820 FQ	LREC Breeding Program	Fiber Quality
12-1-1104 FQ	LREC Breeding Program	Fiber Quality
12-18-314 V	LREC Breeding Program	Verticillium Wilt
12-20-402 N	LREC Breeding Program	Root-Knot Nematode
12-20-407 N	LREC Breeding Program	Root-Knot Nematode
12-20-607 N	LREC Breeding Program	Root-Knot Nematode
12-20-701 N	LREC Breeding Program	Root-Knot Nematode
12-20-1206 N	LREC Breeding Program	Root-Knot Nematode
13-2-501 FQ	LREC Breeding Program	Fiber Quality
13-2-905 FQ	LREC Breeding Program	Fiber Quality
13-2-1005 FQ	LREC Breeding Program	Fiber Quality
13-2-1009 FQ	LREC Breeding Program	Fiber Quality
13-9-218 S	LREC Breeding Program	Abiotic Stress
13-2-913 FQ	LREC Breeding Program	Fiber Quality
13-9-1107 S	LREC Breeding Program	Abiotic Stress
13-11-109 BB	LREC Breeding Program	Bacterial Blight
13-18-203 D	LREC Breeding Program	Abiotic Stress
13-18-310 D	LREC Breeding Program	Abiotic Stress
13-31-219 P	LREC Breeding Program	Abiotic Stress

* TX 65 is an accession from the NCGC; first series of numbers in breeding lines is year of selection, second series is test number or letter, and ending series is row number. 2010 selections from test 'B' are divergent paired selections from fiber elongation study. Other breeding lines were selected in environment for: FQ=Fiber quality, P=Pecos, BB=Bacterial blight, N=Root knot nematode, S=Salinity, D=Drought.

3.2.1 Germination Testing and Experimental Design

Experimental design of the germination tests was a randomized complete block design (RCBD) consisting of 4 replications of treated germination paper, and two groups of control germination paper each with a full complement of treatments. The untreated groups were used to calculate reductions prior to statistical analysis and are not used as effects in the model. Controls were soaked in reverse osmosis (RO) water provided by a custom design RO water system located in the LREC greenhouse complex. Salt treated replications were soaked in a 175 mM L^{-1} solution of BioReagent NaCl, ≥99% pure, prepared in RO water. The concentration of the salt solution was based on previous testing and procedures outlined in the Hemphill methods paper (2006). Commercial cultivars were provided directly from the industrial complex in which they were developed, with the industry's fungicidal seed treatment previously prepared on the seed. Common control lines and proprietary breeding lines had no seed treatment. For each block, cultivars were "planted" at a rate of 20 seed per Anchor Paper Company 10" x 20" heavy weighted germination paper. The towels were placed in 6 35.5 cm x 20.3 cm x 11.8 cm clear plastic storage bins with lids labeled with the corresponding treatment. The storage bins containing the germination towels were placed in the LREC greenhouse complex Enconair Plant Growth Chamber, model SG-30, from Enonair Ecological Chambers Inc. Manitoba, Canada. The growth took place at 29.4° C, 85% RH (relative humidity), and a five-day light exposure. As there was limited space in the storage bins and germination chamber, the 32 breeding lines and accession were separated into 5 groups of experiments, with FiberMax FM 989 included as a reoccurring check for

comparison across the experiments. After the five-day growth period, storage bins were removed from the germinator and germination percent and elongated hypocotyl lengths recorded. A seed was considered germinated if the radicle length was greater than 0.5 cm.

Commercial cultivar germination testing was completed in the preliminary testing phase (December 2014). Testing on proprietary breeding lines was conducted Summer/Fall of 2015. Germination and hypocotyl length expressed as a reduction of percent of the control (same line germinated in RO water) were analyzed by ANOVA and Fisher's Protected LSD using Agrobase 2.1 software. A germination salinity index to attribute for both factors was calculated by:

$$Germination\ salinity\ index = (germination\ \%\ reduction \times 0.5) + (hypocotyl\ \%\ reduction \times 0.5)$$

3.3 HYDROPONIC TESTING

3.3.1 Hydroponic System

Each hydroponic test used six, 76.2 cm x 30.5 cm x 14 cm plastic storage bins. The bins were coated in two to three layers on the exterior of the bin of Rust-Oleum® Paint for Plastic, color white, to prevent excessive light entrance into the root zone of the tubs, and to protect the tubs from break down by ultraviolet (UV) light. The six tubs contained two bubble stones, 35.6 and 25.4 cm in length connected to an Aqua Culture double outlet aquarium air pump by airline rubber tubing. The corresponding sized lids to the tubs had four rows of holes drilled at 1.3 cm diameter for suspension of the young

seedlings and were spaced at 2.54 cm increments. This spacing allowed for a maximum of 80 plants per tub. Each tub was then filled with 30.28 L of RO water. A micronutrient solution was added containing 7 g ammonium nitrate, 1.7 g monoammonium phosphate, 3 g potassium chloride, 537 g calcium nitrate, and 4 g magnesium sulfate. The hydroponic system was allowed an approximate 24 hr. settling period to ensure complete dispersion of the micronutrient solution in the water. Pipette tips, cut to approximately 4 cm in length were used to aid plant suspension.

3.3.2 Initiation of Hydroponic Experiments

Each experiment was initiated with a germination period in the LREC greenhouse complex Enconair Plant Growth Chamber. Seeds were "planted" at a rate of 25 seeds per Anchor Paper Company 10" x 20" heavy weight germination paper soaked in RO water, and placed in 35.5 cm x 20.3 cm x 11.8 cm clear plastic storage bins with lids. Seeds were germinated at 29.4° C, 85% RH, and a five-day light exposure. Commercial cultivars in tests conducted Fall 2014 through Summer 2015 were given a five-day growth period. Commercial cultivars and breeding lines tested in Fall 2015 through Summer 2016 were given a four-day growth period. The time difference was to achieve similar plant development after some greenhouse modifications hastened plant growth. After the growth period in the germination chamber, 10 uniform seedlings were selected for transfer into the hydroponic system.

3.3.3 Hydroponic Experimental Design and Testing

The experimental design for each hydroponic trial was a randomized complete block design (RCBD), consisting of four treated replications, and two control replications. The hydroponic method was modified from the "Screening Method for Salt Tolerance in Cotton" (Hemphill et al., 2006). Preliminary testing indicated too many excessive confounding factors to compare cultivars across runs. To limit the confounding factors, only eight lines could be tested at once in a testing period. Eight cultivars or lines, (Tables 2 and 3) were selected based on results from the germination testing, selecting consistent top, medium and low performers, with FM 989 as the reoccurring check. Ten plants of each cultivar or breeding line were used as the experimental unit size.

Table 2. Commercial cotton cultivars selected for hydroponic salinity screening based on salinity germination rating. *

Variety	**PVP***	**Rating**
PhytoGen PHY 367 WRF	N/A	High
PhytoGen PHY 499 WRF	N/A	High
All-Tex Nitro 44 B2RF	PVP201300460	High
Deltapine DP 1044 B2RF	PVP201000260	Med
NexGen NG 4111 RF	N/A	Med
FiberMax FM 2484 B2F	PVP201200291	Low
FiberMax FM 2989 GLB2	PVP201200130	Low
FiberMax FM 989 (check)	PI603956, PVP9800259	Low

*(USDA, 2017) N/A cultivars are not PVP-applied, and utility patent number is unknown.

Table 3. Cotton accession and LREC cotton breeding lines selected for hydroponic salinity screening based on salinity germination rating

Breeding Line	Pedigree
12-18-314V	(Acala Maxxa x 82-DTT-822-2) x FM 989
6-46-153P	Verhalen V83 x CA 1012
11-11-607BB	{[CA 2266 x (80-NNN-28-1 x Stahman P)] x [Ca 489 x (CA 2268 x Stahman P)] x [MOP-85-FCX-82-14x(CA 2153 CA 3026)]} x Acala Maxxa GTO
12-20-607N	[(EPIg#547-60-611-1)-2-77 x (CA 2268xStahman 05-2B)]x{[(CA 3090xAuburn 634)xEPSM 74-1094-4-76xCA 2267)]x[(CA 3090xAuburn 634)x(EPSM 74-1094-4-76xCA 2267)]}
13-9-218S	[(Stahman P x CA 2266)x CA3027] x TX 307 (PI 165390)
FM 989 (check)	PI603956, PVP9800259
TX 65	PI 154101
12-1-640FQ	FM 958 x (TX 15 x BBB-10)

Each tub contained 30.28 liters of RO water and the micronutrient supplement at the time of the transfer. Commercial cultivars in tests conducted Fall 2014 through Summer 2015 were given a three-day acclimation period, before the addition of BioReagent NaCl, ≥99% pure, in RO water. Sodium chloride was added at a rate of 77.2 g in four-24 hour intervals until 175 mM L^{-1} solution of NaCl was reached. Commercial cultivars and breeding lines tested in Fall 2015 through Summer 2016 were allowed to acclimate to the hydroponic system for one day before the addition of salt. Salt

treatment were added at a rate of 132.7 grams in three-24 hour intervals until a final concentration of 225 mM L^{-1} solution of NaCl was obtained.

An Ultrameter III™ Model 9PTK, produced by Myron L Company, Carlsbad, CA, was used to determine the amount of NaCl dissolved in the water as total dissolved solids (TDS) and to determine the pH of the solution. The pH of the solution was controlled with the General Hydroponics™ pH Up and Down solutions as needed throughout the experiments.

In both testing procedures, the seedlings grew to the third true leaf stage, as determined by the growth stage of the two control replications. At harvest, seedlings were cut along the root apex transition zone. Leaves, shoots, and roots were separated, and individual shoot and root length measurements were taken at the time of harvest. The leaves, shoots, and roots for the 10 individual seedlings were combined and dried in Fisher Scientific Isotemp® Premium Oven Model 55°C. Dry leaf, shoot, and root weights were recorded after seven days in the oven.

To examine the response due to salinity treatment, average fresh shoot and root length and shoot and root biomass of treated vs. untreated plants of each cultivar was compared. Percent of growth in treated hydroponics compared to untreated hydroponics for each cultivar and breeding line was analyzed by ANOVA (Analysis of Variance) and Fisher's Protected LSD (Least Significant Difference) using Agrobase 2.1 software.

3.4 FIELD OBSERVATIONS

Cultivars exhibiting significant differences in the germination evaluation and selected for hydroponic evaluations, were included in field evaluations that exhibited high and low salinity tolerance. Entries were planted in a RCBD with four replications at the Texas A&M AgriLife Research Station in Pecos, TX, in 2015 and 2016, and the Texas Tech University Quaker Avenue Research Farm in Lubbock, TX, in 2016. The soil at Pecos location has a Hoban silty clay loam (fine-silty, mixed, superactive, thermic Ustic Haplocalcid). The Lubbock location soil is an Acuff loam soil type characterized as a fine-loamy, mixed, superactive, thermic Aridic Paleustolls (USDA, 2016). In both locations, a subset of six cultivars was selected for their high, medium, and low rating performance in the germination screenings, and their technology package for management in the Pecos location (Table 4). Standard management practices for each location were utilized in both years.

Table 4. Commercial cotton cultivars selected for field observation at Pecos (2015-2016) and Lubbock 2016 based on salinity germination rating.

Variety	PVP*	Rating
PhytoGen PHY 499 WRF	N/A	High
All-Tex Nitro 44 B2RF	PVP201300460	High
Deltapine DP 1044 B2RF	PVP201000260	Med
NexGen NG 4111 RF	N/A	Med
FiberMax FM 2484B2F	PVP201200291	Low
FiberMax FM 2989GLB2	PVP201200130	Low

*(USDA, 2017) N/A cultivars are not PVP-applied, and utility patent number is unknown.

The Pecos location used one row plots, planted on 0.86-1.07m variable row spacing, the equivalent of 0.97 m row spacing. Plots in this location were 15.2 m long, with an alternating 15.2 m "alley" between plots. This large plot size and alternating design was selected to allow for salinity gradation through the length of the field, and account for possible large spaces caused by Phymatotrichum root rot (Texas root rot), *Phymatotrichopsis omnivorum*. Tests were planted at Pecos on 26 May 2015 and 14 June 2016. The Lubbock location used two rows 9.5 m plots on 1.0 m row spacing, and planted 6 June 2016. In 2016, a heavy rain event at Pecos damaged the field causing a total loss of the Pecos location for that year, though one seedling emergence stand count was conducted on 7 June 2016. Seedling emergence and survival notes were taken on 22 June, 30 June and 8 July, 2015, in Pecos; and 5 July and 12 July 2016 in Lubbock. Only two stand counts were taken at the Lubbock location because there was not a change in emergence counts between the two weeks. Plant height was measured after final node growth was reached, as an average of plants in each plot.

A 50-boll sample was taken from each plot prior to harvest to obtain data on picked lint percent, pulled lint percent, and boll size. Picked lint percent is the lint fraction of seed cotton, pulled lint percent is the lint fraction of the burr cotton, boll size is the weight in grams of seed cotton per boll. Boll samples were de-burred using a two-saw cylinder stick machine and feeder-extractor, and ginned on a ten-saw gin equipped with an incline cleaner, feeder extractor, and saw lint cleaner at LREC.

The 2015 Pecos location was harvested 8 December, and the 2016 Lubbock location was harvested 30 November with a modified John Deere 482 two-row stripper. Cotton from each plot was caught in burlap sacks, then weighed for total plot weight. After weighing, approximately 600g sub-sample was taken from the center of the burlap bag. Samples were ginned on a ten-saw gin equipped with an incline cleaner, feeder extractor, and saw lint cleaner at LREC to obtain lint and seed turnout and apply lint turnout to plot weight to estimate lint yield. A fiber sample of approximately 30 g was taken to obtain fiber data using High Volume Instruments (HVI) with 4 micronaire, and 10 length/strength determinations per sample.

Yield, lint and seed turnout, picked and pulled lint percent, boll size and plant height; and fiber properties micronaire, length, length uniformity, strength and elongation were analyzed via Agrobase 2.1 for both locations.

4. RESULTS AND DISCUSSION

4.1 PRELIMINARY TESTING

4.1.1 Preliminary germination testing

Attempts to modify LREC salinity screening protocols in place since 2006 to phenotype NCGC accessions began in 2012 with an initial aim to detect subtle differences among current commercial cultivars. The first phase of testing was the use of the germination protocol described in materials and methods. Initial germination screening was conducted on commercial and near-commercial cultivars entered in the 2013 and 2014 Official Variety Trails (OVT) in the Plains region of the National Cotton Variety Testing Program (USDA, 2013; USDA, 2014)) and current commercial or near-commercial cultivars that are most often used by producers in the region. In 2013, 35 commercial or near-commercial cultivars were evaluated and 32 cultivars in 2014. Cultivar owners determine which cultivars will be entered in the NCVT trials, with the exception of four national standards, and in 2013 and 2014, five regional standards selected by the NCVT committee. Germination percent and hypocotyl length expressed as a percent of the control were analyzed using Agrobase 2.1 software. Control in each case was a cultivar germinated in RO water treated germination paper; and was compared to the same cultivar germinated in germination paper using salt-treated water to calculate percent of control.

Results from 2013 and 2014 germination data were published in Dever et al. (2014) “Cotton Performance Tests in the Texas High Plains and Trans-Pecos Areas of

Texas (2013)" and Dever et al. (2015) "Cotton Performances Tests in the Texas High Plains (2014) (Appendix Tables 1 & 2). These data were taken as part of a separate OAP project, and were observed in separate years. Significant differences for salinity response were detected among commercial and near-commercial cultivars for both germination percent and hypocotyl length. Cultivars tested both years showed generally similar responses, except for NexGen NG 3306 B2RF, which had a significant difference in performance rank from 2013 to 2014. Though the salinity index dropped for all the cultivars from 2013 to 2014, the cultivars performed similarly when compared to each other. As this was observed as separate years, genotype by year interaction was not analyzed and these data were only used to select cultivars for further testing. The reason for the drop in salinity index between the two years is unknown, but is likely caused by different lots of seed being used from year to year.

4.1.2 Preliminary hydroponic testing

Hydroponic trials were conducted in 2013 and 2014 at the LREC greenhouse complex. Cultivars evaluated were the 35 commercial or near commercial cultivars entered into the 2013 OVT in the Plains region of the NCVT and current commercial or near-commercial cultivars that are most often used by producers in the region.

Based on initial analysis of hydroponic growth data, unexpected and unknown factors appeared to be influencing the results (Fig. 1 – 4). These confounding factors were hypothesized to be different time of year the experiments were conducted, difficulties with maintaining pH level, differences in individual tub environments,

impurities in the salt, or other unknown sources of variation. Some sources of variation contributed to differences observed in the control cultivar, FM 989, used as a reoccurring reference through the six experiments that were required to evaluate all the entries. This information suggested that the timing of the experiments was significant, $P<.0.05$, for root length, shoot length, root biomass, shoot biomass, leaf biomass, and percent reduction of root, shoot, and leaf biomass. Reduction of root and shoot length was highly significant across the six experiments for salt treated water. Hydroponic tub effect was significant for shoot length and percent shoot length reduction, and was highly significant for shoot biomass and percent reduction of root length.

Fig 1. FM 989 shoot length across six different hydroponic salinity experiments conducted in 2013-2014.

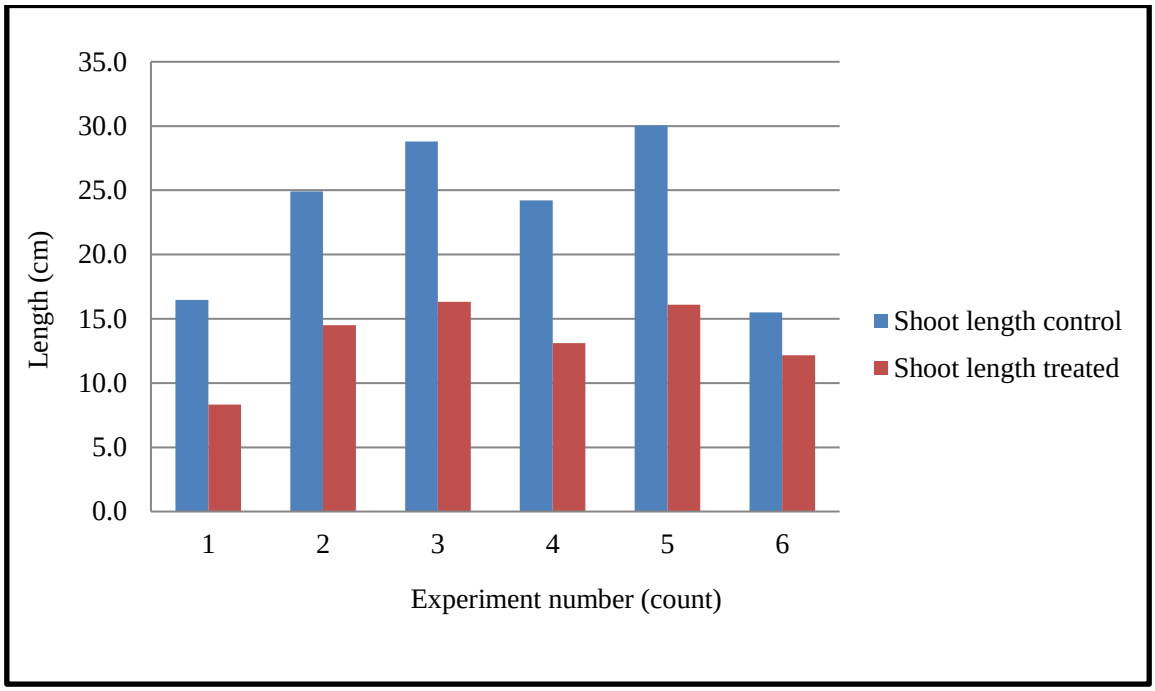


Fig 2. FM 989 Root length across six different hydroponic salinity experiments conducted in 2013-2014.

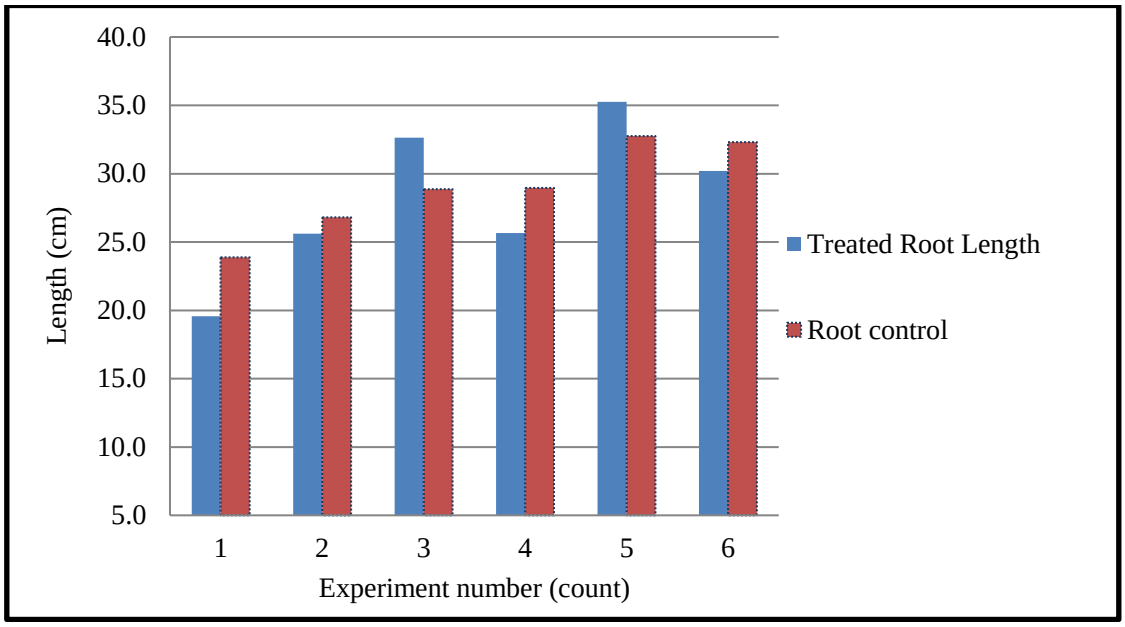


Fig 3. FM 989 dry biomass across six different hydroponic salinity experiments conducted in 2013-2014.

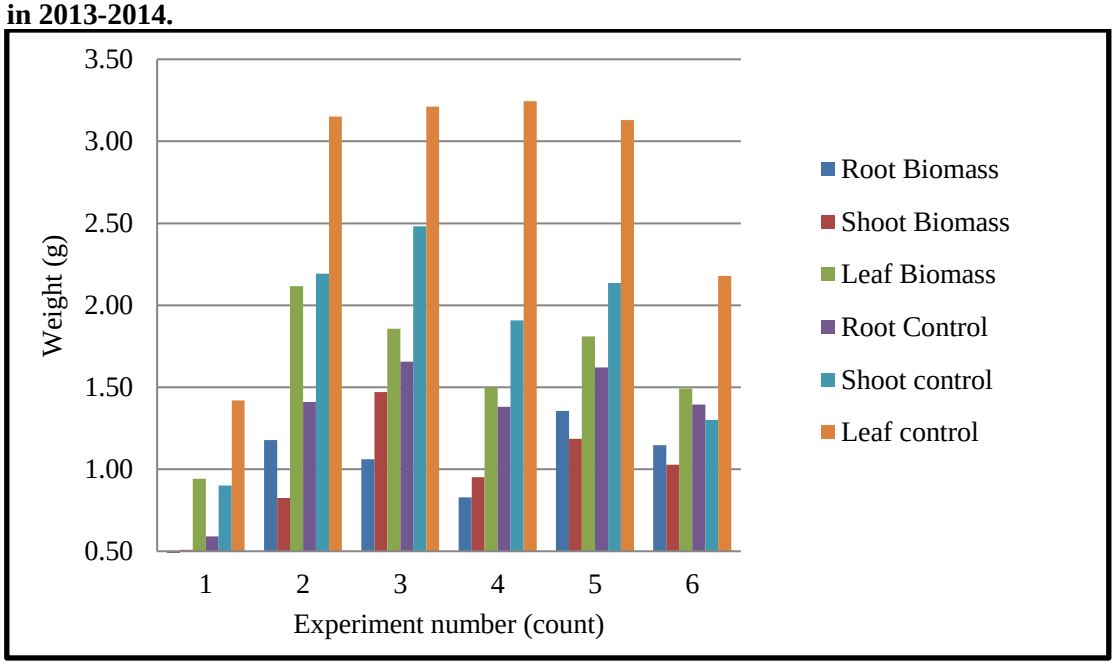
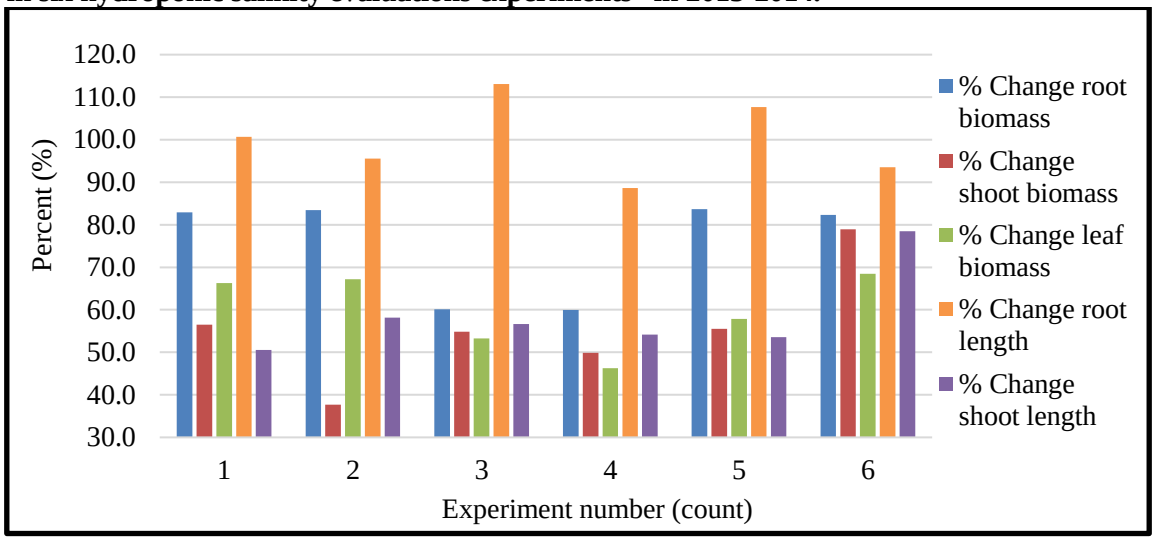


Fig 4. Average percent change in root and shoot length and biomass for FM 989 evaluated in six hydroponic salinity evaluations experiments* in 2013-2014.



*seven commercial cultivars were tested in each experiment

4.2 GERMINATION TESTS ON BREEDING LINES

In 2015, 32 breeding lines, one accession and control FM 989, were evaluated for salt tolerance using the germination screening protocol described for preliminary germination screening. ANOVA showed significant differences for percent reduction in germination percent and hypocotyl lengths (Tables 5 and 6). It was also shown that replication for germination was significant at the 0.05 level. Significant rep effects are usually difficult to explain unless the experiment is established to identify variation within a field or within a greenhouse. In this case, the cause of the significance among the reps is unknown.

Table 5. ANOVA for percent reduction in germination percent from salinity treatment of 34 genotypes tested in 2015.[1]

Source	df	SS	MS	F-value
TOTAL	127	716399.9		
REP	3	2277.511	759.17	4.35*
ENTRY	33	53465.42	1620.164	9.27*
RESIDUAL	91	15896.99	174.692	

*Significant at P<0.05
[1]Data shown with 8 missing observations due to missing plants

Table 6. ANOVA for percent reduction of hypocotyl length germinated in saline versus reverse-osmosis water for 34 genotypes tested in 2015.[1]

Source	df	SS	MS	F-value
TOTAL	129	7053.331		
REP	3	72.962	24.321	0.84
ENTRY	33	4287.179	129.915	4.49*
RESIDUAL	93	2693.191	28.959	

*Significant at P< 0.05
[1] Data shown with 6 missing observations due to missing plants

Percent reduction of the control in germination and hypocotyl lengths are shown in Table 7.

Table 7. Percent reduction in germination percent and hypocotyl length, and salinity index for cotton breeding lines and accession evaluated for germination response in salinity, 2015.[2]

Designation	Salinity Index	Germination Percent (% reduction)		Hypocotyl Length (% reduction)	
FM 989[3]	30.1	9.0	n[1]	51.1	l[1]
FM 989	32.9	7.5	n	58.3	jk
13-9-218S	33.8	7.7	n	59.9	i-k
12-18-314V	35.4	11.9	mn	59.0	jk
10-B-9	38.1	14.9	l-n	61.4	g-k
TX 65	39.8	15.2	l-n	64.4	e-j
12-1-820FQ	41.5	27.5	h-l	55.6	kl
12-1-1104FQ	42.3	27.8	h-l	56.8	kl
12-20-407N	42.5	16.2	l-n	68.8	c-e
13-18-203D	43.1	20.4	k-n	65.9	c-i
23-1-609FQ	43.7	17.6	l-n	69.9	c-e
12-20-1206N	44.1	19.4	k-n	68.8	c-e
6-21-519FQ	45.1	25.8	i-m	64.5	d-j
11-11-505BB	45.5	22.9	j-n	68.2	c-g
10-B-12	45.8	29.7	h-l	61.9	f-k
13-2-913FQ	48.8	30.3	h-l	67.3	c-g
11-11-307BB	48.9	26.9	h-m	70.9	bc
13-13-219	48.9	37.2	f-j	60.7	h-k
6-46-153P	49.2	33.9	g-k	64.5	d-j
12-20-701N	49.2	27.8	h-l	70.6	c-e
10-B-10	49.9	33.4	g-k	66.4	c-h
10-B-11	50.7	34.6	g-k	66.9	c-h
12-1-640FQ	51.8	38.3	f-j	65.4	c-i
13-9-1107	54.6	39.7	f-i	69.5	c-e
12-20-402N	55.0	42.4	e-h	67.6	c-g
11-11-607BB	57.9	46.2	d-g	69.6	c-e
13-11-109BB	59.9	52.7	d-f	67.1	c-g
13-2-905FQ	62.7	58.3	cd	67.1	c-g
11-11-128N	64.7	61.4	b-d	68.0	c-f
13-18-310	67.2	57.4	c-e	77.1	ab
13-2-1009FQ	69.9	69.8	a-c	70.1	c-e
13-2-1005FQ	71.0	76.3	ab	65.8	c-i
13-2-501FQ	74.5	78.2	a	70.8	b-d
12-20-607N	77.9	75.8	ab	80.1	a
Mean		35.1		65.8	
c.v. %		37.7		8.2	

[1]Means followed by the same letter are not significantly different at P<0.05

[2]Salinity concentration of 175 Mm L^{-1}

[3]Results from reoccurring entry FM 989 from two germination runs are included to show consistency between runs

Percent reduction of germination averaged 35.1% for all the breeding lines at 175 mM L^{-1} salinity level with germination reduction ranging from 7.5% to 78.2%. Percent reduction of hypocotyl length averaged 65.8%, and ranged from 51.1% to 80.1%. The breeding line germination test was divided into five "runs" with FM 989 in each of the five runs as a common check. One of the five FM 989 runs had a clerical mistake that was carried over to the Excel file, and two others were dropped due to missing data. The two remaining were used in the analysis to indicate that FM 989 reacted similarly across runs and using FM989 as a reoccurring check was valid.

The reoccurring check cultivar, FM 989, had the least reduction from control (no salinity) stress to salinity stress treatment for both germination and hypocotyl percent, with 7.5% and 9.0% reduction in germination and 58.3% and 51.1% hypocotyl reduction. They were not significantly different from each other, which was expected. Five genotypes were not significantly different than the FM 989 check in percent germination reduction. These were 12-20-1206N, 11-11-307BB, TX 65, 10-b-12, and 13-2-913FQ. Five genotypes, 13-9-218S, 13-2-1005FQ, 13-9-1107, 12-20-407N and 11-11-607BB, exhibited significantly greater reductions in percent germination reduction than all other genotypes tested.

The FM 989 check also averaged the smallest absolute reduction in hypocotyl length (Table 7). 12-1-820FQ, 12-1-1104FQ, 12-18-314V and 13-9-218S all had relatively low reduction in hypocotyl lengths: 55.6%, 56.8%, 59.0%, and 59.9%

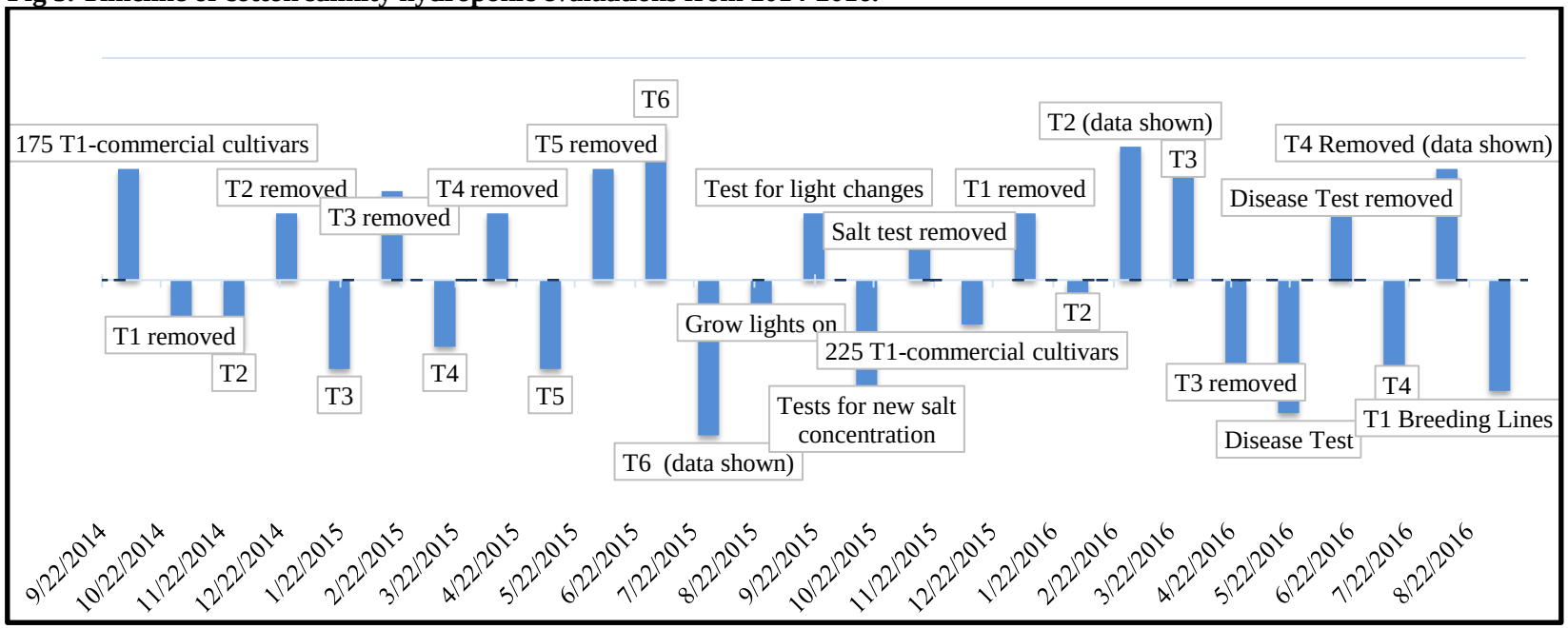
respectively. Breeding lines 12-20-607N, 13-18-310, and11-11- 307BB all had high reduction rates at 80.1%, 77.1%, and 70.9% respectively. While the range in hypocotyl reduction percent was not as drastic as that of germination reduction, significant differences were determined at 95% probability level. From these data, a subset of seven high, medium, and low performing lines was selected to be tested in hydroponic growth screening, along with FM 989.

4.3 HYDROPONIC TESTING

4.3.1 Optimizing hydroponic system

From the preliminary testing, continuous modifications were made to the hydroponic system and the protocol in attempts to reduce environmental factors inhibiting successful completion of the experiments. A total of 11 evaluation experiments were conducted on commercial cultivars and breeding lines, along with four experiments conducted solely for testing new modifications of the hydroponic system (Fig 5). Of the 11 evaluation experiments, only four experiments were considered free enough from mitigating factors to collect reliable data associated with salinity response. The four experiments reported include commercial cultivars evaluated at 175 mM L^{-1} salinity concentration; two experiments evaluating commercial cultivars at 225 mM L^{-1} salinity concentration; and one experiment evaluating breeding lines at 225 mM L^{-1} salinity concentration.

Fig 5. Timeline of cotton salinity hydroponic evaluations from 2014-2016.



Changes to the hydroponic system were minor. The system was painted with UV plastic paint rather than UV sun tape as described in Castillo (2011), as the tape did break down causing a deterioration of the plastic tubs. For safety measures in the greenhouses, an electrical drop outlet was installed, along with water-tight electrical conduit mounted on the tables holding the hydroponic system. The electrical modifications allowed for the use of Aqua Culture double outlet 227L aquarium pumps to be used, providing more adequate aeration. Lack of aeration is commonly the most frequent cause of loss of plants in soilless cultures (University of Hawaii Cooperative Extension Service, 1970).

Modifications of the greenhouse unrelated to the salinity screening project also had a significant impact on the hydroponic evaluations. The greenhouse bay that contained the hydroponic system was modified with Gavita Enhanced 400W HPS lights mounted on CAN 400 aluminum ballasts, for the purpose of increasing the number of plant generations that could be cycled through the greenhouse for other breeding projects. To increase the effectiveness of the grow lights for advancing breeding generations, temperatures were increased from 25-27°C to 28-31°C. The lights and increased temperature decreased the length of time for seedlings growing in hydroponics to reach the third true leaf stage. To account for this decreased time, the protocol was modified to the final increased salinity concentration (225 mM L^{-1}), shorter acclimation period, and shortened salt application protocol.

Throughout testing, environmental factors had significant influence on the success of the evaluations. Several influencing factors were found throughout testing including: calcium precipitating out of nutrient solution, pH maintenance, algae growth, and disease infestation. Exploration for solutions to each of the problems was continually attempted throughout the duration of testing.

Many hydroponic systems are so highly aerated that if pH is not adjusted daily the calcium precipitates out of the solution as insoluble calcium carbonate and the pH rises (Texas A&M AgriLife Extension, 2015). The precipitation of the calcium, as well as the fluctuating pH, was easily controlled by the integration of General Hydroponics™ pH Up and Down solutions and the use of laboratory grade fertilizers. The order in which the fertilizer solution was added contributed to the successful elimination of this problem. For the nutrient solution herein: add ammonium nitrate, monoammonium phosphate, and potassium chloride first; reduce pH to at or below 6.3; finally add calcium nitrate and magnesium sulfate. In many cases the first three fertilizers will lower the pH enough that addition of pH solution is not needed. After the nutrient solution is dispersed throughout the system, the pH may need to be raised to the proper level, approximately 6.3 for these evaluations.

Excessive algae growth was a significant problem in the hydroponic systems. While algae are common- carried in water supplies, on seedlings and old root systems, on equipment, or transported by humans, animals, wind, and, ubiquitous on the Texas High Plains, dust, which makes complete avoidance difficult (Morgan L. , 2008). Since

complete avoidance is difficult, small amount of algae were usually not of great concern. When algae growth is thick and widespread, it becomes a problem by using nutrients, reducing oxygen content, and could possibly suffocate roots (Morgan L. , 2008). Schwarz and Gross (2004), describe the impact of algal growth in hydroponically grown lettuce and report that the algal growth had a negative impact on the growth of the lettuce. There are algicide products for use in hydroponic systems, but most require large quantities and can also have detrimental effects on other plants in the system.

The excessive algae growth in the evaluations conducted for this research was managed in several different ways. Firstly, the implementation of using laboratory grade salt and fertilizer reduced the contamination from those sources. Secondly, the ability to better control pH levels helped delay and decrease the amount of algae in the systems. Finally, hydrogen peroxide was used to provide control of algae from becoming excessive. However, hydrogen peroxide was used sparingly as it does pose a risk to the roots (Morgan L. , 2008). Another option to be explored to prevent the algae from proliferating in a hydroponic system would be to use dark color paint, tape, or other covering to prevent excessive light reaching the nutrient solution.

Once the nutrient solution, pH, and algae problems were addressed, disease infestation became an apparent and most damaging problem in the hydroponic system. Larsen (1982) did not recommend the type of system that we used due to warm weather in Texas, which increases the nutrient solution temperature making plants more susceptible to disease infestation. Disease in hydroponics is usually contributed by

excess organic matter being introduced into the system by un-sanitized systems, dust, remaining plant material or from new plants, and from airborne spores entering the growing space (Song, et al., 2004). Pythium, Phytophthora, and Fusarium species are common problems for hydroponic systems and there are not currently any fungicides registered for commercial hydroponic use (Larson, 1982; Greenhouse Vegetable Information, 2007). In this hydroponic system, the disease was determined to be Fusarium fungus, and experiments were conducted to find a reasonable method to manage the Fusarium. Hydrogen peroxide and chlorine were first used as options to clean the hydroponic system and attempt to prevent the disease from infesting the system. This treatment can require high doses, up to100 ppm of hydrogen peroxide, and at such high doses can damage the roots of the plants (Greenhouse Vegetable Information, 2007). Options for use of a fungicide in the sanitized system to prevent the development of the fungus were also explored. Song et al. (2004) explored chemical options for the control of Fusarium in hydroponic systems, finding the most effective option to be prochloraz and carbendazim fungicides. These fungicides are not labeled for use in hydroponics in the United States, and many fungicides are not labeled for use in greenhouses.

A small experiment was conducted with two fungicides, Trinity® from BASF and Consan 20® from Ferti-Lome®, to determine the effects of these fungicides on Fusarium (BASF, 2017; Ferti-Lome, 2017). Both fungicides are not labeled for commercial hydroponics, but are registered for use in greenhouses. The fungicides were added at the rate labeled for fungicide control in potted plants to the two different hydroponic

systems, with another system not having fungicide treatment. The three systems were brought to the 225 mM L^{-1} salinity concentration, as it was previously observed the disease would contaminate the salt treated tubs before the control tubs. Trinity did prevent the development of the Fusarium longer than Consan 20 or in the non-treated system. Trinity is to be applied every 10 to 14 days, and Consan 20b is to be applied every 7 days (BASF, 2017; Ferti-Lome, 2017). Both fungicides did not completely prevent infestation of Fusarium in the hydroponic system, but treatment application times could be adjusted to manage infestation. Trinity also has the disadvantage of having some plant growth inhibiting properties, which could confound the plant growth measurement analysis.

4.3.2 Hydroponic Evaluation Results

4.3.2.1 Hydroponic evaluation of commercial cultivars at 175 mM L^{-1} salt concentration

Initial hydroponic tests were conducted at a 175 mM L^{-1} salt concentration. For these data, all values were transformed to account for negative values by adding a constant of 100 for all parameters. Analysis of variance showed significant differences between cultivars for percent reduction of control for shoot and root lengths at 175 mM L^{-1} concentration ($P<0.05$) (Table 8). It also showed that replication was significant for both shoot and root lengths, indicating that there were still other factors continuing to affect the hydroponic system.

In some cases plants in salt treated tubs produced longer roots, longer shoots and more biomass than those of the same cultivar in tubs with no salt. This was not expected. Thus, some percentage reductions were negative which makes comparisons confusing. For the purposes of this discussion, only the percent reduction in the parameters measured was used to compare performance of cultivars, not the actual length of roots.

Table 8. ANOVA for fresh shoot and root length reduction of commercial cotton cultivars evaluated in hydroponics at 175 mM L^{-1} salt concentration, August-October 2015.[1]

		Shoot Length			Root Length		
Source	df	SS	MS	F-value	SS	MS	F-value
TOTAL	30	2372.4			28882.1		
REP	3	371.3	123.8	5.3*	11978.6	3992.9	11.1*
GENOTYPE	7	1535.7	219.4	9.4*	9732.8	1390.4	3.9*
RESIDUAL	20	465.4	23.3		7170.7	358.5	

*Significant at $P< 0.05$

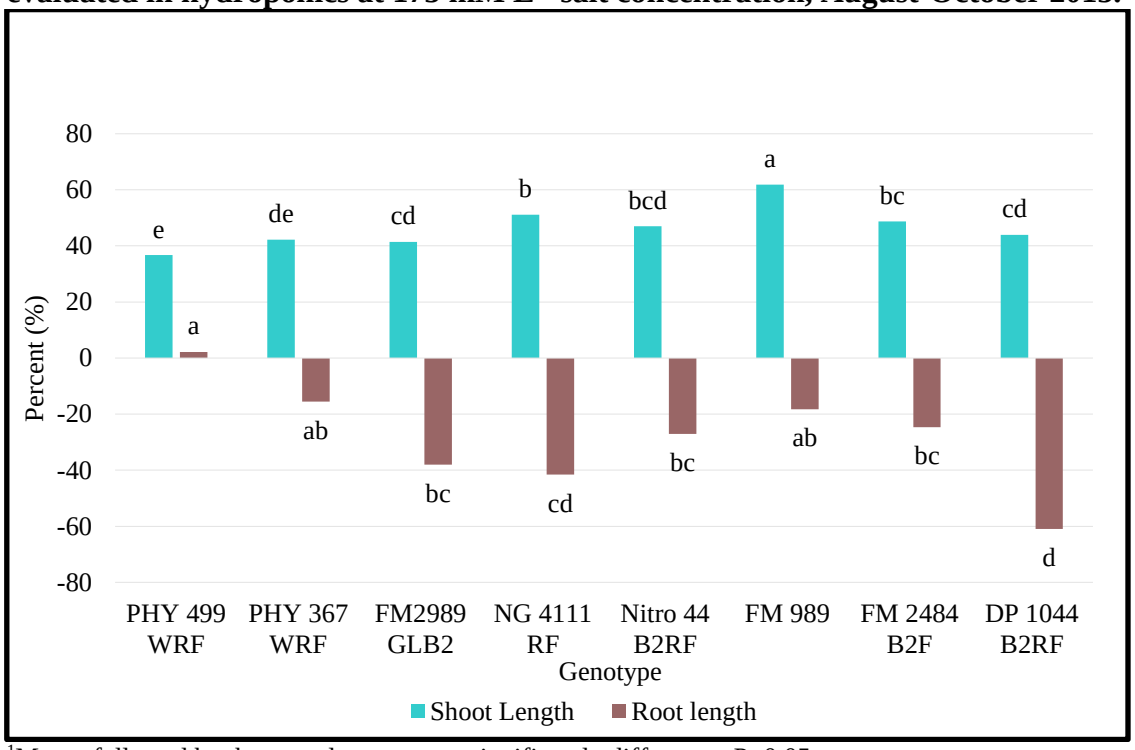
[1]Data shown with 1 missing observation due to missing plants

Average reduction for shoot length was 46.8% and ranged from 36.7% to 61.8% (Fig. 6). PhytoGen PHY 499 WRF had shoot length reduction of 36.7%, with PhytoGen PHY 367 WRF performing similarly with a reduction of 42.2%; these two cultivars also performed well in germination testing. All-Tex Nitro 44 B2RF was rated as a high performer in germination testing, but only had moderate performance in hydroponic screening with a shoot length reduction of 47.0%. FiberMax FM 989 and NexGen NG

4111 RF had the highest shoot length reduction at 61.8% and 51.1% respectively (Fig. 6).

Percent reduction of root lengths averaged across cultivar was 27.7% greater than the control, and ranging from 61.0% greater than control to 2.2% less than control (Fig. 6). Deltapine DP 1044 B2RF had the least root reduction at 61.0% greater than control. Ranking second and third were NexGen NG 4111 RF and FiberMax FM 2989 GLB2 at 41.6% and 38.1% greater than untreated control, respectively. PhytoGen PHY 499 WRF and PhytoGen PHY 367 WRF had the greatest root length reductions at reductions 2.2% less than the control and 15.5% greater than the control, respectively. This was a change in performance rating from germination testing, as two of the high performers in the germination tests performed poorly in the hydroponic test for root length.

Fig 6. Percent reduction of shoot and root lengths of commercial cotton cultivars evaluated in hydroponics at 175 mM L^{-1} salt concentration, August-October 2015.[1,2]



[1]Means followed by the same letter are not significantly different at P<0.05.
[2]Reductions shown are averages before transformation for analysis.

Analysis of variance of dry biomass rates determined significant differences between the cultivars at 175 mM L^{-1} NaCl concentration (Table 9). Replication was significant for shoot and root dry weights, but was not significant for leaf dry weight. Significant replication difference potentially masked meaningful differences between cultivars and indicated further modification to the testing protocol was needed. Root dry weights had an average of 6.3% reduction ranging from 27.3% greater than no salinity stress treatment control to 36.5% less (Fig. 7). Shoot dry weight reduction averaged 41.2% with Deltapine DP 1044 B2RF having the least reduction, 35.6%, and NexGen

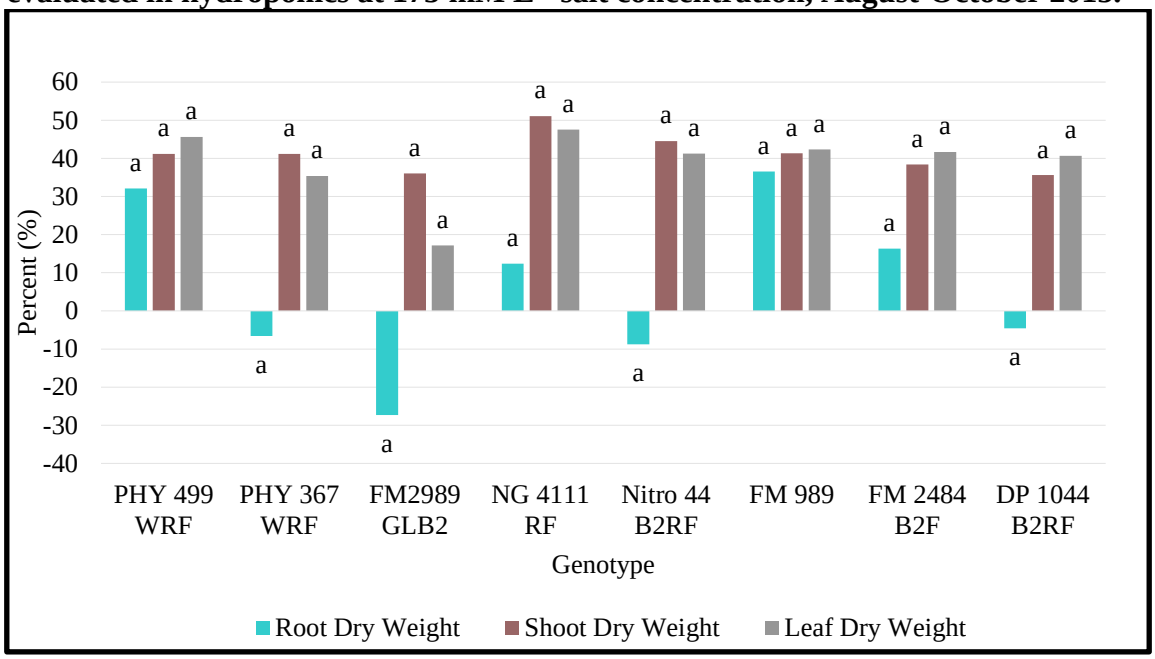
NG 4111 RF with the highest, 51.1% (Fig. 7). Leaf dry weight averaged 38.9% reduction and ranged from 17.1% to 47.5% (Fig. 7).

Table 9. ANOVA for percent reduction of plant biomass weights of commercial cotton cultivars evaluated in hydroponics at 175 mM L^{-1} salt concentration, August-October 2015.

		Root Dry Weight			Shoot Dry Weight			Leaf Dry Weight		
Source	df	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value
TOTAL	31	46417.5			5566.5			6952.9		
REP	3	15800.8	5266.9	6.44*	2742.3	914.1	9.0*	1172.9	391.0	2.5
GENOTYPE	7	13429.9	1918.5	2.34	680.7	97.2	0.9	2533.2	361.9	2.3
RESIDUAL	21	17186.9	818.4		2143.4	102.1		3246.8	154.6	

*Significant at P< 0.05

Fig 7. Percent reduction of plant biomass weights of commercial cotton cultivars evaluated in hydroponics at 175 mM L^{-1} salt concentration, August-October 2015.[1,2]



[1]Means followed by the same letter are not significantly different at P<0.05.
[2]Reductions show are averages before transformation for analysis.

4.3.2.2 Hydroponic evaluation of commercial cultivars at increased 225 mM L^{-1} salt concentration

The modifications for the hydroponic tests included increasing the salt concentration from 175 mM L^{-1} NaCl to 225 mM L^{-1} NaCl, which was used throughout the remainder of the hydroponic screening, with results of two experiments on commercial cultivars reported herein. For these data, no transformations were necessary for analysis. Analysis of variance for the first 225 mM L^{-1} concentration test indicated that there were significant differences between cultivars for both shoot and root lengths (Table 10). Replication was also a significant source of variation.

Table 10. ANOVA for shoot and root length reduction of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, March 2016.

		Shoot Length			Root Length		
Source	df	SS	MS	F-value	SS	MS	F-value
TOTAL	31	3406.6			5393.8		
REP	3	359.3	119.7	6.2*	573.1	191.1	4.5*
GENOTYPE	7	2645.1	377.9	19.7*	3929.8	561.4	13.2*
RESIDUAL	21	402.2	19.2		891.0	42.4	

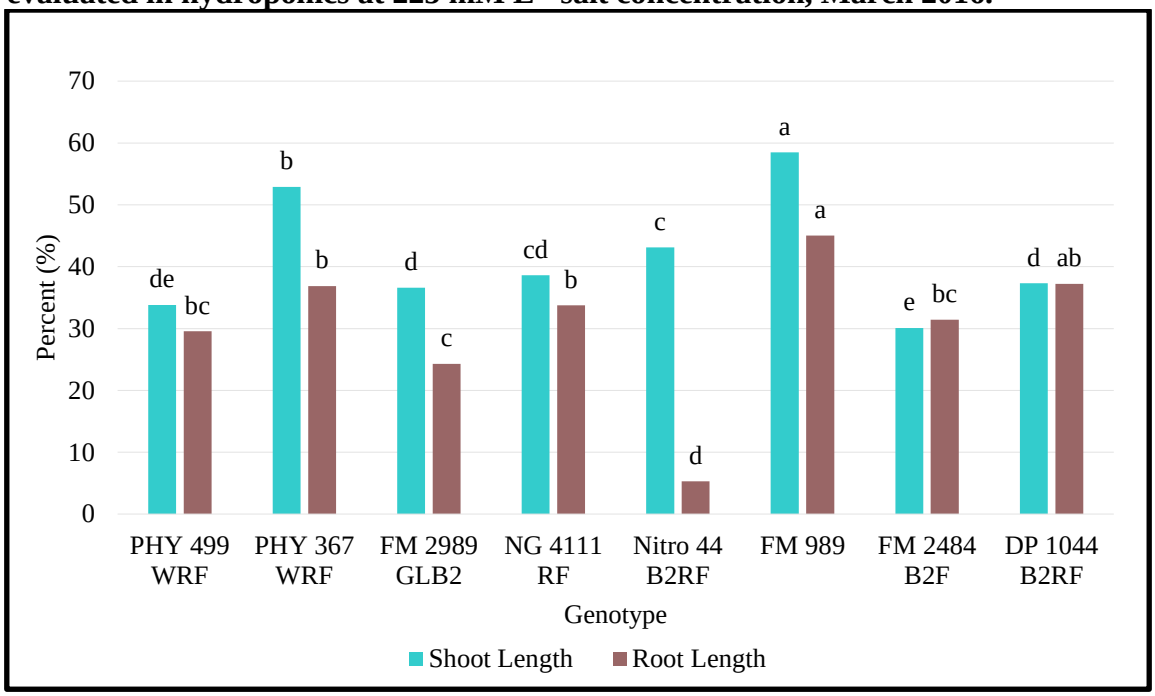
*Significant at P< 0.05

Shoot lengths had an average of 41.4% reduction compared to the untreated control, with a range of 30.1% to 58.5% (Fig. 8). PhytoGen PHY 499 WRF was ranked second with 33.8% reduction, duplicating its rank as a high performer in the germination screening and at the 175 mM L^{-1} NaCl concentration. At this higher concentration, FiberMax FM 2484 B2F had the least reduction in shoot lengths, 30.1%, contradicting its rating from germination screening as a low performer. Also, contradicting its former rating as a high performer in germination and at 175 mM L^{-1} salinity concentration was PhytoGen PHY 367 WRF, which had a reduction of 52.9% at the 225 mM L^{-1} NaCl concentration. FiberMax FM 989 had the greatest reduction in shoot length at 58.5%.

Average reduction for root lengths was 30.4%, varying between 5.3% and 45.0% (Fig 8). PhytoGen PHY 499 WRF was ranked third at 29.6% reduction. All-Tex Nitro 44 B2RF and FiberMax FM 2989GLB2 had the least reduction in root lengths, 5.3% and 24.3%, respectively. FiberMax FM 989 and Deltapine DP 1044 B2RF had the most root

length reduction, 45.0% and 37.2% respectively. PhytoGen PHY 367 WRF also had a high reduction in root length, 36.9%.

Fig 8. Percent reduction of shoot and root lengths of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, March 2016.[1]



[1]Means followed by the same letter are not significantly different at P<0.05.

Analysis of variance for dry biomass showed significant differences between cultivars for root, shoot, and leaf biomass (Table 11). Root dry weight averaged 60.9% reduction, ranging from 48.6% to 81.5% (Fig. 9). Average reduction for shoot dry weight was 54.4% with a range of 38.5% to 76.2% (Fig. 9). Leaf dry weight reduction had an average of 53.7%, 32.2% least reduction to 78.2% highest reduction (Fig. 9).

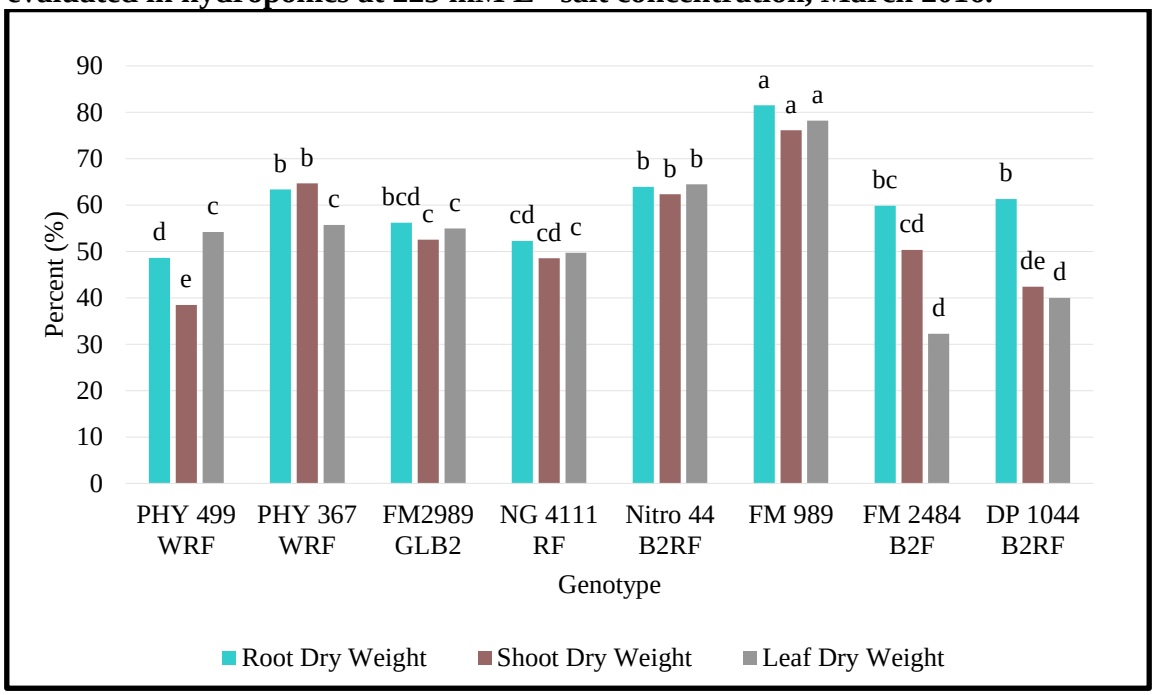
Table 11. ANOVA for percent reduction of plant biomass weights of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, March 2016.

		Root Dry Weight			Shoot Dry Weight			Leaf Dry Weight		
Source	df	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value
TOTAL	31	4807.8			6598.7			6851.0		
REP	3	1023.7	341.2	6.9*	1021.7	340.6	6.0*	321.1	107.1	2.3
GENOTYPE	7	2748.0	392.6	8.0*	4379.1	625.6	11.0*	5554.0	793.4	17.1*
RESIDUAL	21	1036.1	49.3		1197.8	57.0		975.9	46.5	

*Significant at P< 0.05

PhytoGen PHY 499 WRF again out ranked the other cultivars numerically with the least percent reduction in root and shoot dry weight, 48.6% and 38.5%, respectively. NexGen NG 4111 RF and FiberMax FM 2989GLB2 were not different (P=0.05) than PhytoGen PHY 499 WRF in root dry weight reduction, with 52.3% and 56.2% respectively. In reduction of dry leaf weight PhytoGen PHY 499 WRF was moderately ranked with a reduction of 54.2%. At the higher concentration, PhytoGen PHY 367 WRF and All-Tex Nitro 44 B2RF had highest reductions, after control FiberMax FM 989, for all dry biomass measurements dry root weight reduction of 63.4% and 63.9%, shoot weight of 64.7% and 62.4%, and leaf weight reduction of 55.7% and 64.5%, respectively, indicating they did not present the same tolerance as at the 175mM L-1 concentration in the previous hydroponic and germination screenings.

Fig 9. Percent reduction of plant biomass weights of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, March 2016.[1]



[1]Means followed by the same letter are not significantly different at P<0.05

This test was repeated and analyzed separately as attempts were made to mitigate outside influences on the hydroponic system. In this dataset, the values were transformed to account for negative values by adding a constant of 100 for data points of root length and all biomass reductions; shoot length did not require normalization for negative numbers. Analysis of variance for percent reduction of shoot and root lengths revealed significant differences in root length reduction, but shoot length reductions were was not significant between the cultivars (Table 12). Replication was also a significant source of variation for both shoot and root length reduction. This test had a late infestation of disease and plants were harvested before total mortality occurred. The disease could have been a source of variation that masked the effects of the salinity treatment.

Table 12. ANOVA for shoot and root length reduction of commercial cotton cultivars at 225 mM L^{-1} salinity concentration, second data set, May-June 2016.[1]

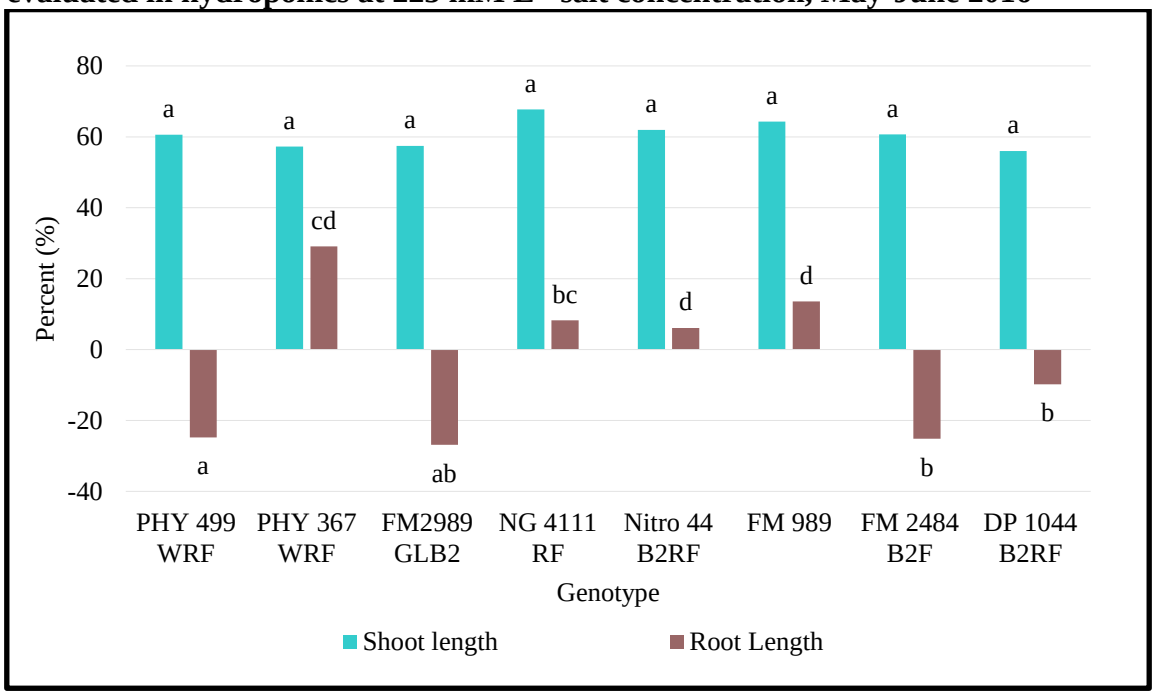
		Shoot Length			Root Length		
Source	df	SS	MS	F-value	SS	MS	F-value
TOTAL	30	2072.6			26485.9		
REP	3	1115.4	371.8	12.6*	10612.2	3537.4	15.3*
GENOTYPE	7	367.8	52.5	1.8	11236.5	1605.2	6.9*
RESIDUAL	20	589.4	29.5		4637.1	231.9	

*Significant at P< 0.05

[1]Data shown with 1 missing observation due to missing plants

Shoot length reduction averaged 61.3% and there were no shoot length reduction differences in values ranging from 56.0% and 67.8% among cultivars (Fig. 10). Root length reduction ranged between 26.8% greater than control to 29.1% less than control with an average of 2.7% less than control (Fig. 10). FiberMax FM 2989GLB2 and FiberMax FM 2484B2F had the least root reduction, 26.8% and 25.1% greater than control, respectively. These cultivars may have had advantage against the disease by either lineage or possibly the seed treatment that was provided industry's fungicidal seed treatment previously prepared on the seed. PhytoGen PHY 499 WRF had moderate performance with a root length reduction of 24.8% greater than control. PhytoGen PHY 367 WRF had a greater root length reduction, 29.1% less than control, compared to the redundant control FiberMax FM 989, which was 13.6% less than control.

Fig 10. Percent reduction of shoot and root lengths of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, May-June 2016 [1,2]



*Means followed by the same letter are not significantly different at P<0.05.
[2]Average reductions before normalization.

Analysis of variance for dry biomass rates determined no significant differences between the cultivars (Table 13). Root dry weights had an average of 50.3% reduction with no significant difference among cultivars ranging from 29.5% (PHY 499 WRF) to 67.7% (PHY 367 WRF) (Fig 11). Shoot dry weights averaged 67% reduction ranging from PhytoGen PHY 499 WRF at 55.7% to All-Tex Nitro 44 B2RF at 73.8% (Fig 11). Leaf dry weight averaged 59.5% reduction and ranged from 48.3% to 77.7% (Fig 11). Analysis of variance also indicates replication was significant for all the biomass parameters. The late infestation of disease likely introduced a source of variation other than cultivar salinity response.

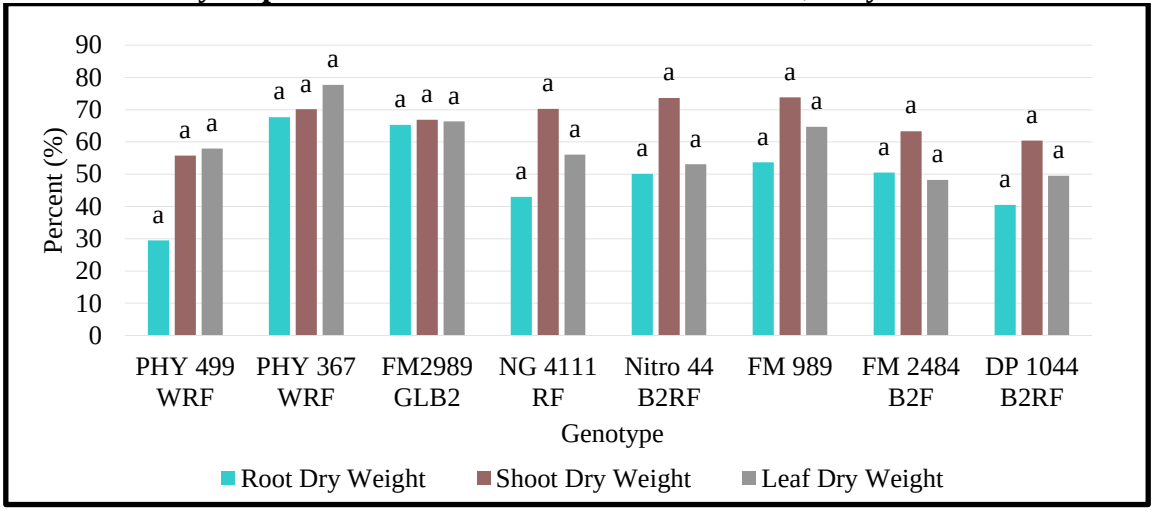
Table 13. ANOVA for percent reduction of plant biomass weights of commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, May-June 2016.[1]

		Root Dry Weight			Shoot Dry Weight			Leaf Dry Weight		
Source	df	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value
TOTAL	30	28194.5			10531.2			13729.3		
REP	3	15235.5	5078.5	11.5*	6311.3	2103.8	12.9*	7705.1	2568.4	13.9*
GENOTYPE	7	4146.9	592.4	1.3	969.6	138.5	0.8	2327.9	332.6	1.8
RESIDUAL	20	8812.1	440.6		3250.4	162.5		3696.3	184.8	

*Significant at P< 0.05

[1]Data shown with 1 missing data point due to missing plants

Fig 11. Percent reduction plant biomass weights for commercial cotton cultivars evaluated in hydroponics at 225 mM L^{-1} salt concentration, May-June 2016.[1,2]



[1]Means followed by the same letter are not significantly different at P<0.05.
[2]Reduction averages before normalization.

4.3.2.3 Hydroponic evaluation results for breeding lines at 225 mM L^{-1} salinity concentration

This hydroponic screening experiment was conducted at the same time as the second commercial cultivar screening experiment, and was also affected by disease infection during the late stages of testing. Analysis of variance of percent shoot and root length reductions did not show any significant differences between breeding lines (Table 14). Replication was significant for root length reduction. Shoot reduction ranged between 7.3% and 40.8%, and averaged 30.4% (Fig. 12). Shoot length reduction of 7.3% for breeding line 6-46-153P and 37.8% for 12-20-607N was not different. Average root length reduction was 8.0% greater than control, and varied between 47.2% greater than

control for breeding line 12-1-640 FQ and 14.8% less than control for line 12-18-314V (Fig. 12).

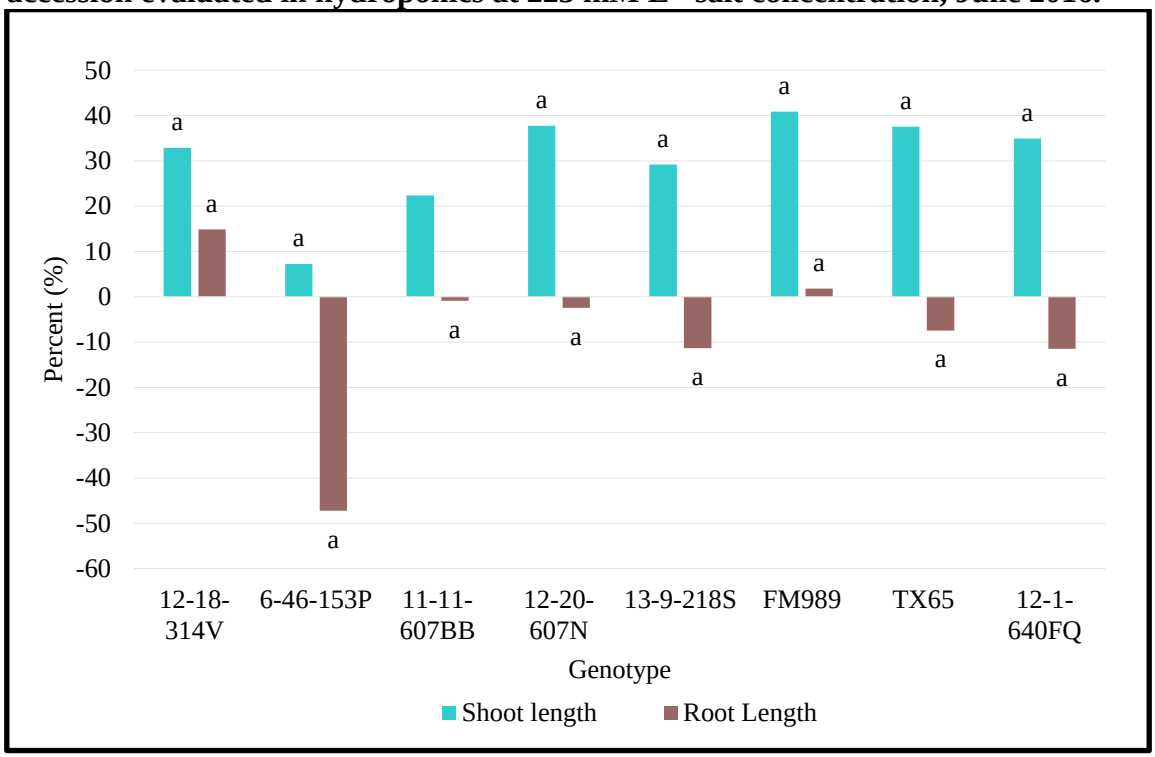
Table 14. ANOVA for shoot and root length reductions of cotton breeding lines and accession evaluated in hydroponics at 225 mM L^{-1} salt concentration, June 2016.[1]

		Shoot Length			Root Length		
Source	df	SS	MS	F-value	SS	MS	F-value
TOTAL	23	7975.6			5265.7		
REP	2	957.0	478.5	1.5	1423.1	711.5	4.6*
GENOTYPE	7	2551.3	364.5	1.1	1653.2	236.2	1.5
RESIDUAL	14	4467.3	319.1		2189.5	156.4	

*Significant at P< 0.05

[1]Data shown with 8 missing observations due to missing plants

Fig 12. Percent reduction of shoot and root lengths of cotton breeding lines and accession evaluated in hydroponics at 225 mM L^{-1} salt concentration, June 2016.[1,2]



[1]Means followed by the same letter are not significantly different at P<0.05.
[2]Reduction averages before normalization.

Plant dry biomass analysis of variance showed significant differences in reduction between lines for all parameters (Table 15). Analysis also indicated replication as a significant factor for root and shoot dry weight reduction, but was not significant for leaf dry weight.

Root dry weight reduction averaged 2.7% greater than control, and reductions ranged between 64.4% greater than control to 33.5% less than control (Fig. 13). Accession TX 65 and breeding lines 12-20-607N, 12-18-314V, and 11-11-607BB had greater root dry weight reduction, 33.5%, 33.1%, 13.1%, 12.5% less than control,

respectively. Lines 6-46-153P, 13-9-218S, and 12-1-640FQ had the least reductions, 64.4%, 19.7%, and 17.6% greater than control, respectively. Shoot dry weight reduction averaged 22.6%, and ranged between 52.6% greater than control to 52.9% less than control (Fig. 13). TX 65 had greater reduction, 52.9%, than FiberMax FM 989, 47% less than control. Lines 12-20-607 N and 6-46-153P had the least shoot dry weight reduction, at 58.6% and 4.6% greater than control, respectively. Average reduction for leaf dry weight was 9.3%, ranging from 29.1% greater than control to 31.8% less than control. Lines 12-20-607N and 6-46-153P had the least leaf dry weight reductions, 29.1% and 22.3% greater than control, respectively. Lines 12-1-640FQ and 11-11-607BB had the greatest reductions, 21.9 and 21.5% less than control, respectively, compared to the other breeding lines and accession evaluated.

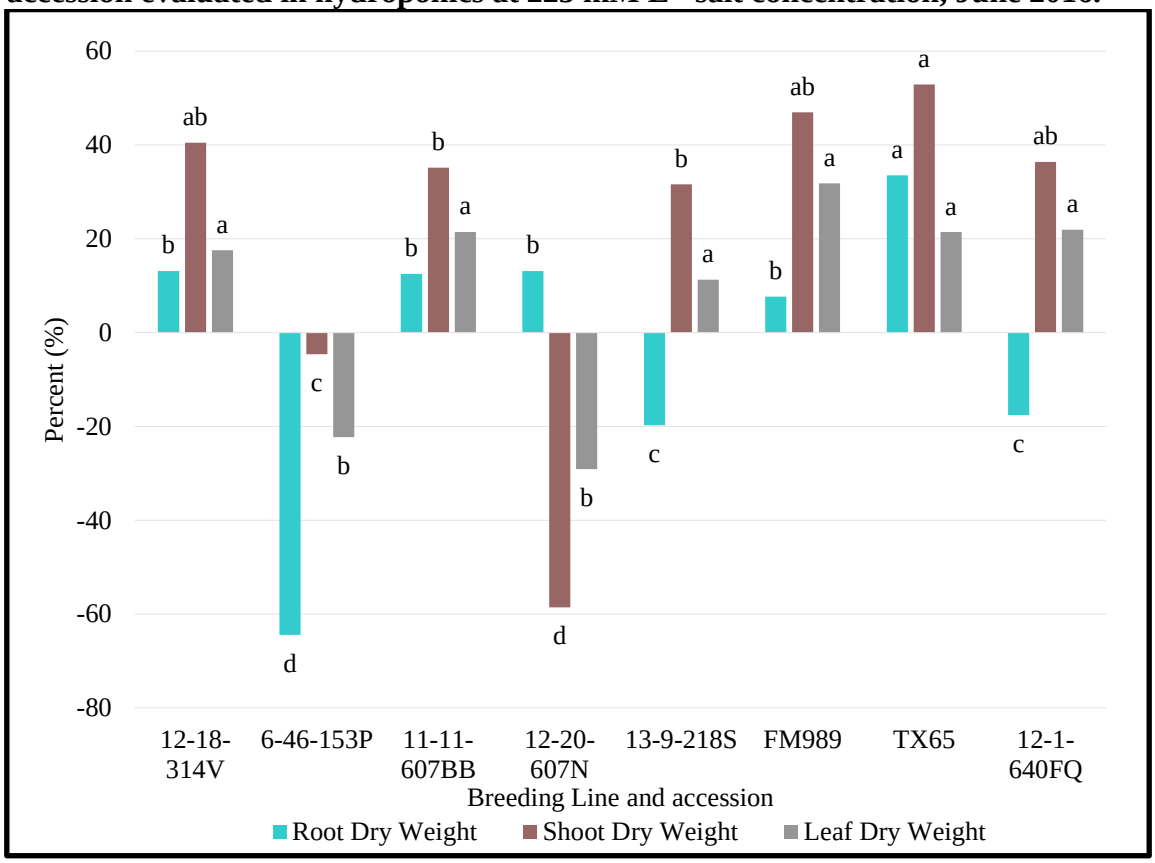
Table 15. ANOVA for percent reduction of plant biomass weights of cotton breeding lines and accession evaluated in hydroponics at 225 mM L^{-1} salt concentration, June 2016.[1]

		Root Dry Weight			Shoot Dry Weight			Leaf Dry Weight		
Source	df	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value
TOTAL	23	26573.9			36067.1			15426.6		
REP	2	4595.7	2297.8	12.6*	5700.9	2850.462	22.1*	1703.6	851.8	3.7
GENOTYPE	7	19423.0	2774.7	15.2*	28556.323	4079.5	31.6*	10528.3	1504.0	6.6*
RESIDUAL	14	2555.2	182.5		1809.863	129.3		3194.7	228.2	

*Significant at P< 0.05

[1]Data shown with 8 missing observations due to missing plants

Fig 13. Percent reduction plant biomass weights for cotton breeding lines and accession evaluated in hydroponics at 225 mM L^{-1} salt concentration, June 2016.[1,2]



[1]Means followed by the same letter are not significantly different at P<0.05.
[2]Reduction averages before normalization.

4.4 FIELD OBSERVATIONS

Six commercial cultivars were evaluated in saline (2015-2016) and non-saline (2016) field conditions to attempt to compare germination screening results with field performance. Evaluations were conducted at the Texas A&M AgriLife Research Station in Pecos, TX (2015-2016), and at the Texas Tech University Quaker Avenue Research Farm in Lubbock, TX (2016). For each location, germination and stand establishment

counts were taken at the beginning of the season, and agronomic and fiber data were recorded at the end of the season (Tables 16-19). Germination and stand establishment observations did not show any differences among cultivars and is not presented herein.

The 2015 observations at the Pecos location indicated PhytoGen PHY 499 WRF was the highest yielding cultivar, 1291 kg ha^{-1}, and also performed well in preliminary germination screenings (Table 16). The two FiberMax cultivars, FM 2484B2F and FM 2989GLB2, were also relatively high yielders at the Pecos location, 1141 and 955 kg ha^{-1} respectively (Table 16). Deltapine DP 1044 B2F had the lowest yield, 755 kg ha^{-1}. While these results were informative, without non-saline field comparison, it was unknown if these data could be attributed to association between germination in the presence of salt, and field performance in saline conditions, or if results were cultivars' response to other factors of the environment.

In 2016, evaluations were planted in non-saline, Lubbock, TX; and saline, Pecos, TX; locations. The two planting locations were selected to determine if initial results from 2015 were the germination association to performance or if the results were cultivar response to environment. At the Lubbock location, PhytoGen PHY 499 WRF, 2359 kg ha^{-1}, was out yielded by both FiberMax FM 2989GLB2 and FiberMax FM 2484B2F, 2380 and 2361 kg ha^{-1} respectively (Table 18). Deltapine DP1044 B2F was the lowest yielder at 2131 kg ha^{-1} (Table 18).

Due to loss of the Pecos location, was not collected from both locations in 2016. The two locations differ in other ways besides soil salt content and only observations are reported; no inference regarding salinity response can be reported.

Table 16. Yield and agronomic property results from cotton cultivar field observations at Pecos, TX (saline conditions), 2015

Cultivar	yield* (k ha^{-1})	lint turnout (%)	seed turnout (%)	picked lint fraction (%)	pulled lint fraction (%)	boll size* (g seed cotton)	height (cm)
PHY499WRF	1291	32.1 a[1]	43.9 c[1]	44.2 a	34.8 a	4.9	63.5 a
FM 2484B2F	1141	31.4 a	44.0 c	42.0 b	33.2 b	4.2	48.3 c
FM 2989GLB2	955	29.6 bc	44.9 bc	40.2 c	31.1 cd	5.2	58.4 ab
NG 4111B2F	873	30.0 b	47.0 a	40.5 bc	31.4 c	4.2	50.8 c
All-Tex Nitro-44B2RF	864	28.9 c	45.3 b	37.7 d	29.6 d	4.7	48.3 c
DP 1044 B2F	755	27.9 d	42.5 d	39.7 c	31.0 cd	4.4	55.9 b
Mean	980	30.0	44.6	40.7	31.8	4.6	53.3
c.v.%	12.7	2.1	2.0	2.2	2.5	7.7	9.1

[1]Means followed by the same letter are not significantly different at P<0.05

*Means were not significantly different at P<0.05

Table 17. Fiber property results from cotton cultivar field observations at Pecos, TX (saline conditions), 2015

Cultivar	mic (unit)		length (mm)		uniformity (%)		strength (kNm kg^{-1})		elongation (%)		leaf (grade)	
PHY499WRF	4.6	a[1]	27.18	c[1]	82.0	ab[1]	300.9	bc[1]	9.8	a[1]	3	c[1]
FM 2484B2F	4.1	c	28.45	b	81.0	cd	291.1	cd	7.8	c	2	a
FM 2989GLB2	4.2	c	27.69	c	80.5	d	285.2	d	7.4	c	2	b
NG 4111B2F	4.4	b	27.69	c	82.2	a	309.7	ab	8.8	b	2	c
Nitro-44B2RF	4.0	cd	29.21	a	81.7	a-c	320.5	a	8.7	b	6	d
DP 1044 B2F	3.9	d	27.18	c	81.4	bc	283.2	d	9.9	a	3	b
Mean	4.1		27.94		81.4		297.9		8.7		3	
c.v.%	3.5		1.5		0.7		3.9		6.7		38.8	

[1]Means followed by the same letter are not significantly different at P<0.05

Table 18. Yield and agronomic property results from cotton cultivar field observations at Lubbock, TX (non-saline conditions), 2016

Cultivar	yield* (kg ha^{-1})	lint turnout* (%)	seed turnout* (%)	picked lint fraction* (%)	pulled lint fraction* (%)	boll size* (g seed cot)	Height* (cm)
PHY 499 WRF	2358	30.4	41.7	43	31.4	4.9	83.8
FM 2484B2F	2360	29.3	41.1	41.1	34.2	5.3	83.8
FM 2989GLB2	2380	28.5	42.4	39.3	30.6	6	78.7
NG 4111 B2F	2332	29.3	43.2	37.7	27.9	5.1	61.0
Nitro-44 B2RF	2232	28.6	43	39.5	30.1	5.4	73.7
DP 1044 B2F	2131	29.2	43.3	39.2	30	4.7	68.6
Mean	2270.7	29.2	42.4	39.4	30.7	5.2	76.2
c.v.%	8.64	4.43	2.78	3.38	5.13	9.38	13.67

*Means were not significantly different at P<0.05

Table 19. Fiber property results from cotton cultivar field observations at Lubbock, TX (non-saline conditions), 2016

Cultivar	mic* (unit)	length (mm)	uniformity* (%)	strength (kNm kg-1	elongation (%)	leaf (grade)
PHY499WRF	4.6	27.18 b[1]	82.0	300.9 a-c[1]	9.8 ab	3
FM 2484B2F	4.1	28.45 a	81.0	291.1 bc	7.8 d	2
FM 2989GLB2	4.2	27.69 b	80.5	285.2 c	7.4 cd	2
NG 4111B2F	4.4	27.69 b	82.2	309.7 a	8.8 ab	2
All-Tex Nitro-44B2RF	4.0	29.21 a	81.7	320.5 ab	8.7 bc	6
DP 1044 B2F	3.9	27.18 b	81.4	283.2 d	9.9 a	3
Mean	4.1	27.94	81.4	297.9	8.7	3
c.v.%	3.5	1.5	0.7	3.9	6.7	38.8

[1]Means followed by the same letter are not significantly different at P<0.05

*Means were not significantly different at P<0.05

5. REVIEW AND CONCLUSIONS

There are various techniques developed to evaluate salt tolerant cotton genotypes. Current and previous research has determined that salinity has a significant effect on the germination of cotton seed, by reducing germination and seedling vigor (Sattar et al., 2010; Bibi et al., 2016; Chachar, Q., 2008; Bhandari, B., 2015; Basal et al., 2006; Ahmad et al. 2002; Kent & Lauchli, 1985; Noor et al., 2001; Abbas et al. 2011). In this study, germination percent and hypocotyl lengths were used as a base for cultivar and breeding line selection for further testing. Initial field study data on cultivars selected from germination screenings seem to indicate results in the germination studies could be predictive of field performance in saline conditions. PhytoGen PHY 499 WRF performed well in germination studies, and was also the highest yielder in saline soil conditions at Pecos. The second year of field study in the saline conditions at Pecos was lost, and inferences cannot be made regarding the association between germination performance in saline water and cultivar yield in saline soils. PhytoGen PHY 499 WRF performed well in salinity germination evaluations, and yielded well in Pecos in the first year of this study, but germination as the indicator of salinity resistance can be misleading per Chachar et al. (2008).

Because of spatial and temporal variation in soil salinity across the field, hydroponic methods have been widely used for salinity screening. Hydroponic techniques have the advantage of being rapid and reliable options to salinity screening (Aktar et. al., 2010; Munns et al., 2002). Aktar et al. (2010) also reported that

hydroponic and soil based screening are both effective in screening cotton genotypes for salinity tolerance. In contrast, Tavakkaoli et al. (2012) reported that hydroponic evaluations did not replicate field conditions, because seedlings were only exposed to the salt for a short period of time. Castillo (2011) found that the hydroponic method was effective for finding phenotypic responses, selection tool for individual plants, but was not effective for selection among adapted genotypes. Bhandari (2015) found limitations in the hydroponic technique and that the addition of salt before the first true leaf resulted in salt shock to the plant causing a significant plant mortality rate for the plants.

Phenotypic and physiological criteria are the main ways that salinity tolerance is characterized (Higbie et al., 2010). The reduction of plant height under saline conditions could be an easy, reliable, and non-destructive way to measure salinity tolerance, as the impact on plant height is prominent (Higbie et al., 2010). However, Abbas et al. (2011) reported percent reduction of dry shoot weight was a more viable method for determining salinity tolerance.

In this study, the use of the hydroponic technique was unreliable. PhytoGen PHY 499 WRF was high performer in the germination screening, at the 175 mM L^{-1} concentration, and in the 225 mM L^{-1} test for root and shoot length and dry biomass reductions. However, results could not be accurately repeated in further evaluations with the commercial cultivars or among breeding lines. While plant performance should be the same throughout a greenhouse, the hydroponic protocol and the system apparently was too easily affected by environmental factors, and the impact of salinity could not be

effectively isolated. The limited number of genotypes that could be screened effectively, and the impacts of the nutrient solution, pH, algae, and disease made use of this screening technique for improved salt tolerance cotton breeding burdensome.

Germination testing was effective in providing information on cultivars or breeding lines to be selected for further salinity testing. It also seemed to be effective in selecting for saline field testing; however, comparisons could not be made in this series of experiments. This should be addressed in further testing. Some preliminary results indicate seed lot, or differences in seed quality, could impact salinity stress germination response. Care should be taken in future research to normalize seed quality among genotypes to be tested in salinity screening experiments.

The preliminary data from the hydroponic tests showed that this particular hydroponic system is effective for examination of physiological responses to salinity, but was not effective for detecting differential response of cultivars or breeding lines to salinity. Collecting data using the hydroponic system is labor intensive and challenging in that it appears to be affected by many factors which may not be controllable. The system in this series of experiments was highly sensitive to environmental changes and was susceptible to disease infestation. For this, and possibly other hydroponic systems, there is a need for approved fungicides that can be used to good effect. For detecting small changes in plant response, other systems such as a pot or sand culture system could provide a better alternative.

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APPENDIX

A-1. Salinity index, germination percent reduction and hypocotyl length reduction for cotton breeding lines and accession evaluated for germination response in salinity, 2013 (Dever et. al. 2014).

Designation	Salinity Index	Germination Percent (% reduction)		Hypocotyl Length (% reduction)	
NGX 3305B2RF	80	102.5	a	57.8	a
NG3306B2RF	77	99.9	ab	54.2	ab
HQ 210CT	72	92.3	abcdef	50.8	abc
Nitro 44B2RF	71	92.5	abcde	49.0	bcde
PHY 725RF	68	95.0	abcd	40.0	fghi
PHY 499WRF	67	95.0	abcd	38.6	fghij
UA 48	66	82.5	bcdefghi	50.3	abcd
PHY 367WRF	65	87.5	abcdefg	42.9	defg
UA 222	65	96.1	abc	33.3	ijkl
PHY 375WRF	65	87.2	abcdefg	42.1	efgh
NG 2051B2RF	65	94.6	abcd	34.6	hijkl
PHY 339WRF	64	77.5	cdefghij	51.1	abc
DP 1321B2RF	62	79.5	cdefghij	44.8	cdef
NG 4111RF	61	85.5	abcdefgh	35.9	ghijk
DG 12353B2RF	60	69.2	ghijkl	50.6	abc
ST 5458B2F	60	86.3	abcdefg	33.2	ijkl
ST 5288B2F	59	75.7	defghij	42.5	efg
DP 1219B2RF	58	80.8	bcdefghij	35.9	ghijk
NG 4010B2RF	54	79.0	cdefghij	29.5	klmno
DP 1044B2RF	54	74.4	efghijk	33.2	ijkl
DP 0912B2RF	53	70.0	ghijkl	36.1	ghijk
NG 1511B2RF	52	73.1	fghijk	31.8	jklm
ST 4946GLB2	51	69.5	ghijkl	33.3	ijkl
NG 3348B2RF	50	68.4	ghijkl	32.1	jklm
FM 9180B2F	50	72.1	ghijk	27.6	lmnop
FM 9250GL	48	66.3	hijklm	29.5	klmno
NG 4012B2RF	47	61.6	jklm	33.3	ijkl
FM 9058F	47	64.5	ijklm	29.9	klmn
AT Epic RF	45	56.3	klm	32.8	ijklm
FM 2011GT	44	48.6	mno	39.9	fghi
FM 2989GLB2	37	52.5	lmn	21.9	op
DG 13125B2RF	30	34.6	no	25.2	mnop
NGX 2306B2RF	27	30.9	o	23.5	nop
FM 2484B2F	27	30.6	o	23.3	nop
FM 1944GLB2	25	29.7	o	21.0	p

A-1 continued.

Designation	Salinity Index	Germination Percent (% reduction)	Hypocotyl Length (% reduction)
Mean		73.2	36.9
c.v. %		18.9	14.8
LSD 0.05		19.4	7.7

*Means followed by the same letter are not significantly different at p>.05

A-2. Salinity index, germination percent reduction and hypocotyl length reduction for cotton breeding lines and accession evaluated for germination response in salinity, 2014 (Dever et. al., 2015).

Designation	Salinity Index	Germination Percent (% reduction)		Hypocotyl Length (% reduction)	
DP 1219 B2RF	80	98.4	a*	60	a*
Jacco	72	97.2	a	45.7	b
PHY 499WRF	70	98.7	a	41	bcde
SSG HQ 210 CT	69	94.5	ab	41.9	bcd
DP 0912 B2RF	67	94.7	ab	39.7	bcde
PHY 725 RF	66	93.6	ab	39.2	bcdef
Nitro-44B2RF	65	88.9	ab	41.7	bcd
DP 1321B2RF	65	82.9	abc	46.3	b
PHY 367WRF	63	91.7	ab	34.5	bcdefg
PHY 222WRF	62	88.7	ab	34.2	bcdefgh
NG 1511B2RF	61	83.3	abc	38.9	bcdef
NG 4111RF	55	78.6	abcd	30.5	cdefghi
SSG UA222	54	73.5	bcde	34.6	bcdefg
PHY 339WRF	48	59.5	defgh	35.4	bcdefg
CT 13442B2RF	47	51.4	fghi	42.8	bc
PHY 333WRF	47	54.5	efgh	38.1	bcdef
Acala Glandless	47	63.3	cdefg	31.2	cdefghi
STV Glandless	45	65.3	cdef	24.6	ghijkl
DP 1044B2RF	39	38.2	hijk	38.8	bcdef
CT 14515B2RF	38	41.9	ghijk	33.8	bcdefgh
ST 4946GLB2	35	44.9	fghij	24.8	ghijk
FM 1944GLB2	29	30	ijkl	28.5	efghijk
NG 3306B2RF	27	30	ijkl	24.7	ghijkl
FM 2011GT	26	20.8	klmn	29.7	defghij
FM 1830GLT	26	29.6	ijkl	21.7	hijkl
FM 1320GL	24	27	jklm	20.5	ijkl
FM 2334GLT	21	14.9	lmn	26.7	fghijk
FM 2484B2F	14	6.4	mn	21.5	hijkl

A-2 continued.

Designation	Salinity Index	Germination Percent (% reduction)	Hypocotyl Length (% reduction)
FM 4747GLB2	13	8.8 lmn	17.2 jkl
FM 9250GL	13	8.8 lmn	16.5 kl
FM 2322GL	9	4.9 n	11.9 l
Mean		56.9	32.8
c.v. %		32.6	33.2
LSD 0.05		21.8	12.8

*Means followed by the same letter are not significantly different at p>.05